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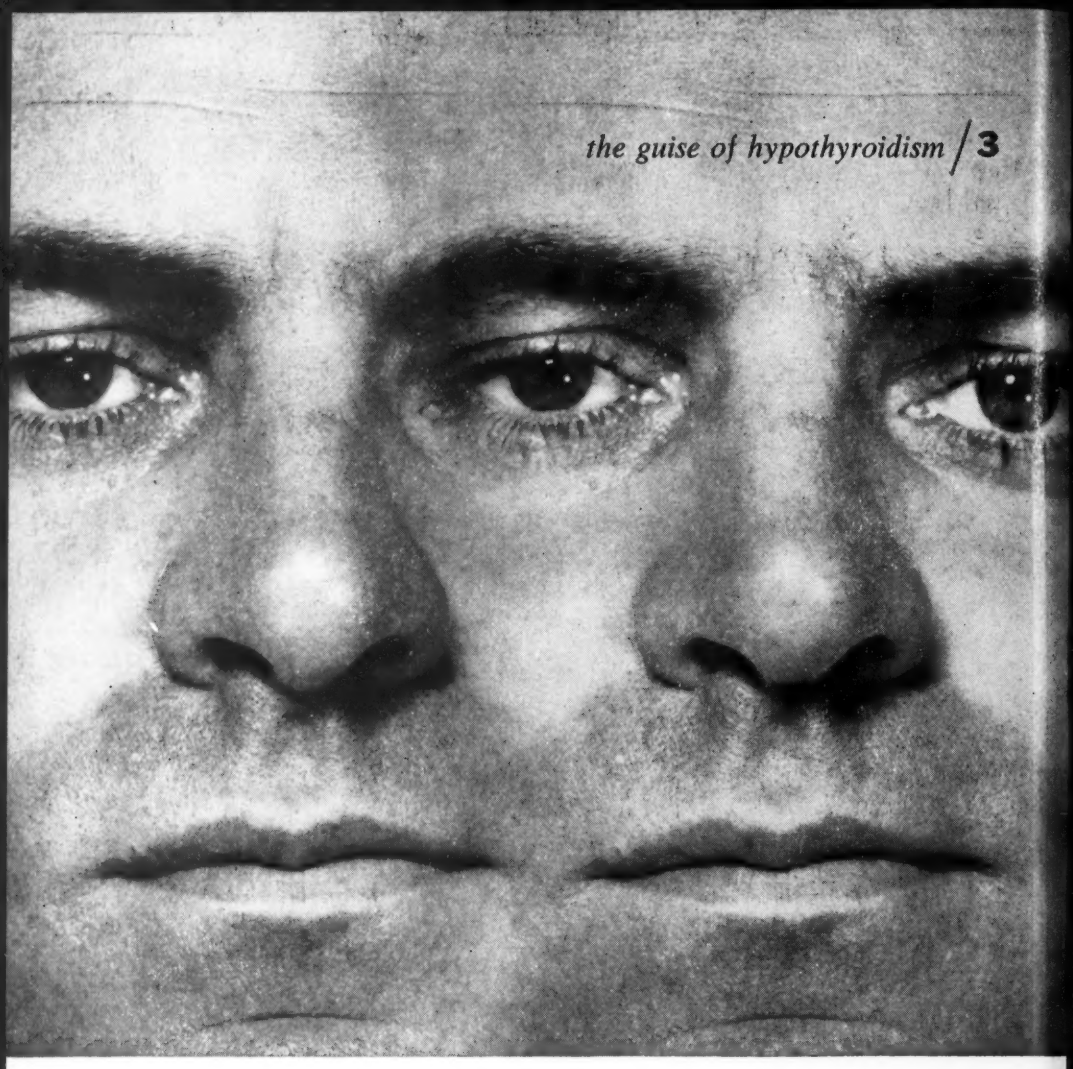

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EFFECT OF ORCHIECTOMY UPON CHEMICAL CONSTITUENTS OF BLOOD IN YOUNG MATURE MALES, WITH SPECIAL REFERENCE TO SUSTAINED INCREASE IN THE LEVEL OF SERUM INORGANIC PHOSPHORUS*

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NINE young adult males were studied for periods as long as two years before surgical castration and four years after removal of the testes. Thus control and experimental data were referable to the same individuals.

The subjects were young mature males, apparently normal with regard to sexual maturation, and in good health throughout the study. Orchiectomy was carried out for medico-legal reasons, not as a therapeutic measure because of illness such as prostatic cancer. Therefore these data, un-

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It is also a pleasure to acknowledge the technical aid of Miss Vada Hazen and the kindnesses of Drs. John D. Crawford and Nathan Talbot in furnishing the data in Table 3, of Dr. Lawson Wilkins for the data in Table 4, and of Dr. Roberto Escamilla for the material in Table 5.

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like those in most previous attempts to assess the effects of castration (1, 2), are not complicated by the lowering of ketosteroid titers that accompanies aging (3–10) or disease (11).

MATERIALS AND METHODS

The 9 subjects were inmates of a home for the feeble-minded, but in many instances their difficulties stemmed chiefly from their social behavior. In only 2 men (M.J. and R.M.) was the I.Q. in the range characteristic of imbeciles.

All subjects followed an unvarying routine with regard to time of awakening, eating and sleeping. During the day all but 2 worked at jobs related to maintenance of the institution, such as farming, dairying, and housekeeping. It was not possible to carry out balance studies, but all these men were served the same food and were studied at the same general intervals after castration. They were castrated at the same time of the same year, only four days intervening between operations on the first and last subjects.

The ages and body weights at the time of castration are listed in Table 1.

TABLE 1. AGE AND BODY WEIGHT OF 9 SUBJECTS AT THE TIME OF CASTRATION

Subject	Age (yrs.)	Body wt. (Kg.)
R. C.	18	—
H. B.	19	57.6
K. C.	19	61.9
R. M.	19	64.1
T. R.	20	41.5
W. B.	22	53.9
R. F.	23	—
M. J.	32	61.7
L. M.	38	78.6

Samples of blood were collected under basal conditions on from two to ten occasions during the two years before castration, and on fourteen occasions afterwards. Calculations of the mean values for the precastrate state were restricted, however, to data obtained during the four weeks immediately preceding orchiectomy.

Values at intervals after castration were compared only with those obtained in the same subject before castration. Changes are referred to as statistically significant if P (derived from Fisher's *t*-test) was .05 or less.

Whole blood was used in measurements of hemoglobin (12), glucose (13, 14), pyruvic acid (15), and lactic acid (16).

Serum was used for measurements of total protein and albumin (17) (with values for globulin calculated from these data), sodium (18), potassium (19, 20), calcium (21), inorganic phosphorus (22), total cholesterol (23), total lipid phosphorus (24), uric acid (25), creatinine (26), and cholinesterase (27).

RESULTS

General. A statistically significant elevation of the serum inorganic phosphorus level occurred after castration and was maintained thereafter.

Significant shifts, limited to a brief period after orchiectomy and attributed at least in part to the surgery, occurred in the levels of glucose, pyruvic acid, uric acid and creatinine.

Items which followed an erratic course, with differences that occasionally reached a significant degree but were not sustained during the four years of study included the concentration of whole blood hemoglobin, lactic acid, serum calcium, total cholesterol, lipid phosphorus, sodium and potassium.

No significant alterations were observed at any time after castration in

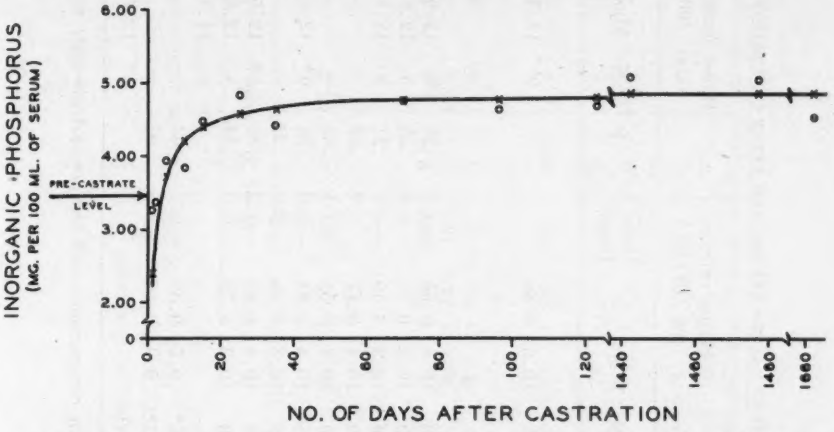

FIG. 1. Sustained increase in mean values for SIP after castration, as studied in blood obtained under basal conditions from 9 young adult males. The increase was statistically significant by the fifteenth day following orchiectomy and remained so thereafter.

Circles represent mean values. The curve was fitted to the observed data by the hyperbolic equation,

$$y = \frac{x}{0.322 + 0.206x}$$

where y is SIP and x the number of days after orchiectomy. This equation yielded minimal residuals, and its variation from the observed data was less than that in successive blood samples from the same subject.

the concentration of total proteins, albumin, globulin and cholinesterase.

Mean values for each item under study, and the statistical analysis, are presented in Table 2. Values for individual subjects are shown only with respect to serum inorganic phosphorus (Fig. 2).

Serum inorganic phosphorus (SIP). The most pronounced alteration after castration, and the only one sustained to a statistically significant degree, was an increase in the level of serum SIP (Fig. 1). The mean value for the 9 subjects prior to castration was 3.45 mg. per 100 ml. After a

TABLE 2. COMPARISON OF MEAN VALUES BEFORE AND AFTER CASTRATION

	Serum inorganic phosphorus (mg./100 ml.)					Serum calcium (mg./100 ml.)					Blood hemoglobin (Gm./100 ml.)			Serum uric acid (mg./100 ml.)		
	N	Mean	σ	Incr.(+) Decr.(−)	P	N	Mean	σ	Incr.(+) Decr.(−)	P	N	Mean	P	N	Mean	P
Before castration	9	3.46	0.584	—	—	9	10.6	0.48	—	—	9	13.3	—	9	4.88	—
Days after castration																
1	9	3.26	0.329	−0.20	.39	9	10.3	0.65	−0.3	.29	9	12.6	.09	9	7.18	<.001
2	9	3.37	0.443	−0.09	.71	9	10.0	0.55	−0.6	.03	9	12.3	.02	9	6.30	.01
5	9	3.94	0.444	+0.48	.07	9	10.3	0.49	−0.3	.21	9	12.7	.28	9	5.21	.35
10	9	3.84	0.770	+0.38	.25	9	10.4	0.77	−0.2	.52	—			9	5.26	.28
15	9	4.48	0.575	+1.02	.003	9	10.7	0.54	+0.1	.68	—			9	4.66	.60
25	8*	4.83	0.432	+1.36	<.001	8*	10.4	0.61	−0.2	.54	6*	12.6	.30	8*	4.63	.73
35	9	4.41	0.432	+0.95	.001	9	10.3	0.49	−0.3	.21	—			9	4.80	.84
70	9	4.75	0.640	+1.29	<.001	9	10.4	0.41	−0.2	.36	8*	12.5	.16	9	4.67	.60
96	9	4.64	0.352	+1.18	<.001	9	10.3	0.55	−0.3	.24	8	12.6	.20	9	4.93	.90
123	5*	4.69	0.547	+1.11	.01	—					2	11.1	.09	5*	5.28	.43
1,442	5*	5.08	0.609	+1.48	.008	5*	9.7	0.39	−0.7	.04	—			5*	4.24	.35
1,477	5*	5.04	0.428	+1.78	<.001	5*	9.9	0.16	−0.5	.07	—			5*	4.38	.16
1,662	4*	4.53	0.442	+1.12	.02	—					4*	12.4	.05	—		

* At intervals when tests were made on less than 9 men, comparisons with the pre-castrate state were limited to values obtained in the *same* subjects prior to orchiectomy.

TABLE 2—*Continued*

	Serum creatinine (mg./100 ml.)			Blood glucose (mg./100 ml.)			Blood pyruvic acid (mg./100 ml.)			Blood lactic acid (mg./100 ml.)			Serum total cholesterol (mg./100 ml.)		
	N	Mean	P	N	Mean	P	N	Mean	P	N	Mean	P	N	Mean	P
Before castration	9	1.19	—	9	89.0	—	9	1.10	—	9	13.2	—	9	177	—
Days after castration															
1	9	1.60	.004	9	76.2	.002	9	1.80	.004	9	8.3	<.001	9	178	>.90
2	9	1.21	.87	9	82.7	.10	9	1.74	.01	9	13.4	.87	9	161	.40
5	9	1.18	>.90	9	72.2	<.001	9	1.61	.02	9	14.2	.60	9	202	.30
10	9	1.24	.70	—			—			—			9	185	.70
15	9	1.27	.52	—			—			—			9	184	.70
25	8*	1.19	>.90	6*	70.3	<.001	6*	1.27	.70	6*	16.8	.007	8*	200	.24
35	9	1.19	1.00	—			—			—			9	162	.39
70	9	1.14	.66	7*	84.1	.08	7*	1.29	.52	7	15.7	.03	9	202	.07
96	9	1.16	.83	9	84.8	.22	9	1.24	.45	9	12.0	.23	9	253	.02
123	5*	0.80	.13	—			—			—			5*	216	.48
1,442	—			—			—			—			5*	238	.07
1,477	—			—			—			—			5*	248	.09
1,662	—			4*	85.2	.67	—			—			4*	228	.43

TABLE 2—*Continued*

	Serum lipid phosphorus (mg./100 ml.)			Serum sodium (mEq./L)			Serum potassium (mEq./L)			Serum total proteins (Gm./100 ml.)			Serum total cholinesterase (units)†		
	N	Mean	P	N	Mean	P	N	Mean	P	N	Mean	P	N	Mean	P
Before castration	9	9.47	—	9	144	—	9	4.84	—	9	6.56	—	9	2.08	—
Days after castration															
1	9	7.81	.14	9	143	.53	9	3.92	<.001	9	6.55	>.90	9	2.05	.86
2	9	8.21	.12	9	144	1.00	9	4.71	.50	9	6.57	>.90	9	1.90	.29
5	9	9.03	.60	9	142	.25	9	4.42	.009	9	6.51	.80	9	2.06	>.90
10	9	9.26	.80	9	142	.26	9	4.46	.03	9	6.57	>.90	9	2.03	.79
15	9	9.43	>.90	9	142	.17	9	4.80	.80	8*	6.72	.62	9	2.01	.68
25	8*	11.05	.02	8*	144	1.00	7*	4.35	.05	7*	6.61	>.90	8	2.16	.79
35	9	9.76	.77	9	146	.19	9	4.09	.001	8*	6.58	.75	9	2.16	.65
70	9	9.64	.86	9	148	.02	9	4.46	.11	8*	6.84	.10	9	1.96	.53
96	9	9.50	>.90	9	145	.56	9	4.59	.16	7*	6.86	.08	9	1.99	.60
123	5*	7.44	.07	—			—			4*	6.61	.84	5*	2.07	.007
1,442	5*	9.62	.16	—			—			3*	7.02	.19	5*	2.26	.54
1,477	5*	9.77	.88	—			—			—			5*	2.04	.46
1,662	4*	8.95	.17	—			—			—			—		

† One unit equals the number of ml. N/100 acetic acid released by 0.5 ml. of serum from 25 mg. of acetycholine chloride in ten minutes at pH 7.8 and 37° C.

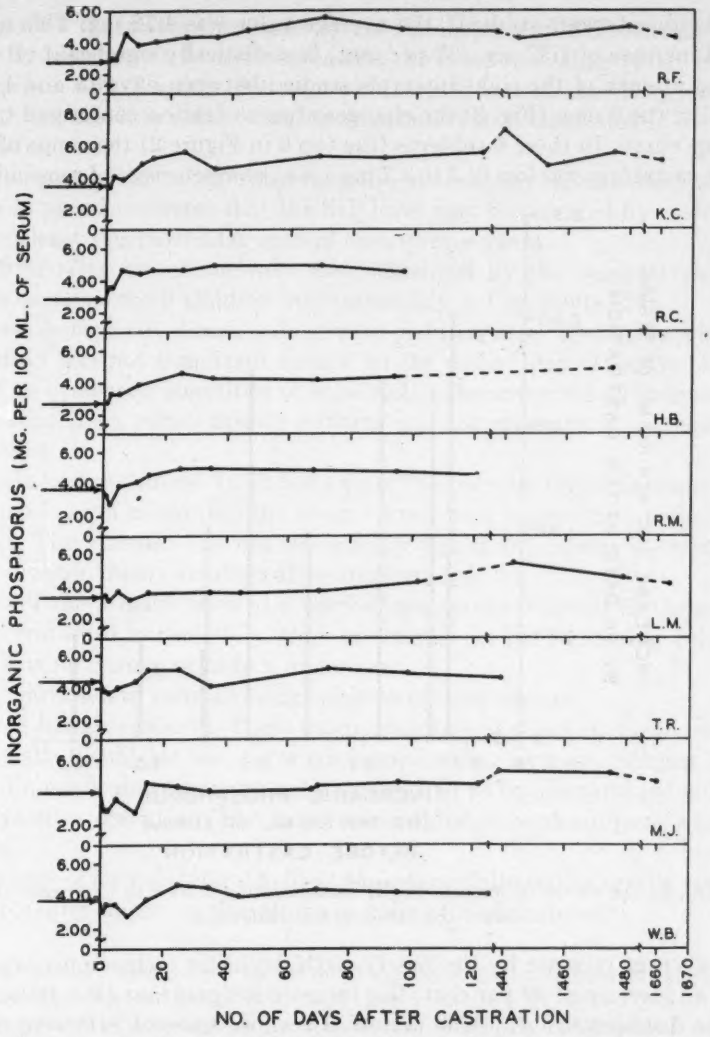

FIG. 2. The effect of castration upon the SIP level in individual subjects.

temporary decrease on the day following orchiectomy, which was observed in 4 of the 9 subjects and was probably attributable to the effects of surgery, the mean value for the 9 subjects returned almost to the preoperative level on the second day (3.37 mg.). By the fifth day after operation the average titer was higher than before orchiectomy (3.94 mg.). From fifteen to 1,662 days after removal of the testes (omitting the forty-fifth day when

only 3 subjects were studied), the average value was 4.72 mg. This maintained increase of 1.37 mg. (37 per cent) is statistically significant (P = .01 or less) at each of the eight intervals studied between days 15 and 1,477.

In 6 of the 9 men (Fig. 2) the changes after castration conformed to the average curve. In these 6 subjects (the top 6 in Figure 2) the range of SIP before castration was low (2.7 to 3.7 mg.), *i.e.*, characteristic of most adults.

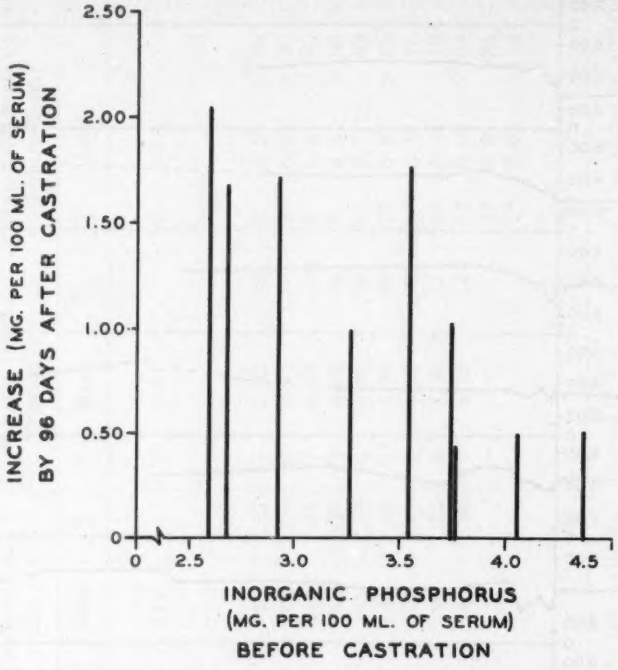

FIG. 3. The extent of increase in SIP concentration tended to be greater the lower the values before castration.

The average increase by the ninety-sixth day after orchiectomy was 1.54 mg., an increase of 49 per cent; this increase is significant (P = .001).

The 3 subjects (T.R., M.J. and W.B.) whose titers of SIP were not increased in a significant and sustained fashion (mean increase of 0.5 mg.) tended to have rather high values prior to castration (3.8 to 4.4 mg.).[1]

[1] In 3 other males who were available for only limited study and who had high SIP levels (means of 4.8, 5.1, and 4.1 mg./100 ml.) before castration at 16, 20 and 23½ years of age, respectively, SIP concentration decreased to the higher levels of the normal range (means of 3.6, 4.2, and 3.9 mg./100 ml.), as measured at one interval after castration, namely at two or three years. The limited nature of this study and the young age of 1 subject reduce the reliability of these results, but these data again indicate that SIP concentration does not increase significantly after castration in all subjects, especially if the values are high prior to orchiectomy.

Figure 3 illustrates the tendency for the increase in SIP concentration after orchiectomy to be more extensive, the lower the values before castration ($r = -.662$, $P = .05$). The degree of increase bore no necessary relationship to the absolute values which were attained after castration ($r = +.193$, $P = .62$).

The range of age of the subjects while under study as castrates, namely 18 to 42 years, indicates that the SIP level may be elevated by castration over at least this particular span of twenty-four years.

Values after castration were not influenced by the seasonal changes which occur in small children but apparently not in adults (28).

Blood hemoglobin. Mean values were reduced after castration but the reduction was not significant except on the second day ($P = .05$). A tendency to decreased quantities of hemoglobin characterized all individuals. The variation in values among subjects was not consistently increased or decreased.

Serum total cholesterol. In all but two of the thirteen intervals when tests were made after castration the mean values were higher than before castration. The increase was not statistically significant, however, except in one test made ninety-six days after orchiectomy.

Two of the 9 males showed a marked temporary decrease in the serum cholesterol level immediately after castration. In the remaining subjects there was no change or only a minor one.

The variation in values among subjects did not change.

Serum lipid phosphorus. There was no maintained change in mean values. In 8 of the 9 subjects the curve conformed to the average. Subject R.C showed a maintained increase until studies had to be discontinued ninety-six days after operation. The variation in values among subjects was not altered.

The *ratio of total cholesterol to lipid phosphorus* followed an erratic course, corresponding chiefly to alterations in values for cholesterol.

Serum calcium. Mean values did not differ significantly after orchiectomy except for low values on the second day ($P = .03$) and again in one ($P = .04$) of two tests after an elapse of four years. The decrease in mean values in tests made four years after castration is probably not meaningful because only 5 subjects were available for study; these 5 men had relatively low values before orchiectomy and there was essentially no change after castration. All individual curves showed a general conformity to the curve of mean values.

As examined in each subject, the changes in calcium and phosphorus following castration showed no consistent tendency to be similar in degree or to be either directly or inversely related to one another. Among the 9 subjects the range in variation in values was not affected in a sustained fashion.

Serum uric acid. Except for a temporary increase ($P = .01$–$.001$), presumably due to the operation, mean values remained unchanged. In 8 of the 9 subjects the curve conformed to the average. In the remaining person (R.F.) the level was not increased but

was reduced and remained so. The variation in values among subjects tended to be slightly but not significantly less immediately after the operation (P = .08 on day 1; P = .20 on day 2).

Serum creatinine. A transient increase occurred in all subjects on the first day (P = .04), followed by an immediate return to the pre-castrate level in all but 1 subject. This status was maintained thereafter in tests covering ninety-six days. In 8 of the 9 subjects the curve conformed to the mean values; lower titers were observed in Subject R.F. The variation in values among subjects became slightly but not significantly less (P = .20).

Blood glucose. Significantly lower values were observed on days 1, 5 and 25. The reduction at days 2, 70, 96 and 1,662 was not statistically significant. In 7 of the 9 men the curve conformed to the mean values. In the remaining 2 men, the levels increased in R.F. and fluctuated in R.M. The variation in values among the subjects was not consistently different.

Blood pyruvic acid. Mean values increased immediately, then began to decrease by the second day but were still significantly higher on the fifth day (P = .02). Individual curves differed considerably from the average. Variation in values among subjects was markedly reduced (P = .001).

Blood lactic acid. Mean values were reduced significantly the first day after castration (P = .001). Reduction was observed in all subjects. Titers returned to pre-castrate levels by the second day; they were significantly higher at two intervals thereafter but measurements for individual subjects were highly variable.

Serum sodium, potassium, total proteins, albumin, globulin, ratio of albumin to globulin, and cholinesterase. Values for these items were not influenced significantly by castration, and the variation in values among subjects was not altered.

DISCUSSION

Definitive influence of castration upon SIP. In most young adults a notable sequela of castration is an elevation of the SIP level that cannot be attributed to hemoconcentration (inasmuch as there was no significant change in the level of serum proteins or of the fifteen other blood elements studied in our series). The present finding that serum calcium concentration does not change is supported by the normal values observed in another series (29) of 11 adult eunuchs in whom only post-castrate values were measured (apparently in a single sample of blood withdrawn at unstated intervals after orchiectomy). Hence, elevation of the SIP level after castration is neither the result nor the cause of changes in the serum calcium level. A lack of direct or close correlation between values for SIP and serum calcium has also been found in certain other states with a high SIP concentration, *i.e.*, childhood (30) and acromegaly (31).

Persistence of high values for SIP for at least as long as four years after removal of the testes cannot be interpreted as a temporary phenomenon associated with transition to a castrate state. It is extremely unlikely that a transition would continue at a rate sufficiently unchanging to cause a plateau-like elevation of SIP between days 15 and 1,662 (Fig. 1). It is concluded that in many, but not in all[1] young adults SIP concentration

TABLE 3. FASTING SERUM VALUES FOR CALCIUM AND PHOSPHORUS IN 12 SUBJECTS WITH UNTREATED HYPOPITUITARISM WHO WERE SEXUALLY IMMATURE AND RETARDED IN BODY GROWTH

(The mean values were 4.27 mg./100 ml. for phosphorus and 9.2 mg./100 ml. for calcium.)

All data in this table were furnished by Drs. John D. Crawford and Nathan Talbot of the Massachusetts General Hospital in Boston, who indicate that the values for SIP in hypopituitarism are, if anything, higher than in control subjects.

Hypopituitarism	Subject		Serum		Manifestation*
	Age	Sex	Ca	P	
Acquired	17	M	9.0	4.2	1, 2, 4, 6, 12
	17		—	4.2	
Congenital, idiopathic	7	M	11.4	3.5	1, 2, 3, 4, 5, 8, 9
	11		9.3	5.5	
Intrasellar craniopharyngioma	14	M	9.8	4.8	1, 2, 3, 4, 6, 12
Intrasellar craniopharyngioma	12	F	9.6	4.8	1, 2, 3, 4, 5, 11
Congenital, idiopathic	14	M	—	4.7	1, 2, 3, 4, 6, 9, 10
Suprasellar pinealoma	5	F	10.0	4.3	1, 2, 3, 4, 7, 12
	10		10.0	5.6	
Congenital, idiopathic	14	M	9.5	4.2	1, 2, 3, 4, 5, 6
Congenital, idiopathic	9	M	8.7	4.6	1, 3, 4, 9
Congenital, familial	11	M	9.8	2.8	1, 3, 4, 9, 11
Intrasellar craniopharyngioma	14	F	9.6	3.9	1, 2, 3, 4, 5, 6, 11
Acquired; thrombosis	13	F	10.4	3.7	1, 2, 3, 4, 6, 7
	15		—	3.8	
Congenital, familial	12	M	9.0	4.4	
	12		—	4.3	1, 2, 3, 4, 6, 8
	13		—	3.8	

* 1 = Linear growth retardation.
2 = Hypothyroidism; PBI <4.0 gamma per 100 ml.
3 = Failure of linear growth acceleration during treatment with thyroid.
4 = Failure of sex maturation by age 16 unless otherwise indicated.
5 = Fasting hypoglycemia.
6 = Abnormally low 17-KS excretion— <1.0 mg. at age 12 years or more.
7 = Abnormally low 11-OCS excretion— <0.2 mg. per sq. meter per twenty-four hours.
8 = Insulin sensitivity.
9 = Testosterone resistance.
10 = Cortisone hypersensitivity.
11 = Positive results with "water" test.
12 = Diabetes insipidus.

quickly becomes stabilized at a new level that characterizes the castrate state.

Inverse relationship of SIP level to gonadal secretions and sex hormones,

TABLE 4. FASTING SIP VALUES IN CASES OF RETARDED OR ACCELERATED GROWTH

The data were furnished by Dr. Lawson Wilkins of The Johns Hopkins Hospital. It would appear that values for SIP are not abnormal either in children who fail to grow normally or those with precocious maturation of primary or secondary sex organs.

Diagnosis	No. of subjects	Age range (yrs.)	Serum P (mg./100 ml.)	
			Range	Avge.
Hypopituitary dwarfism with sexual infantilism	12	10–22	3.4–5.8	4.5
Primordial dwarfism with sexual infantilism	4	4–12	4.4–5.0	4.7
Gonadal dysgenesis	7	10–22	4.7–5.3	4.8
Congenital cretinism	6	3–16	4.2–4.9	4.5
Sexual precocity and accelerated growth	10	4–13	2.6–6.2	4.9

and the influence of age upon this relationship. Two concepts can now be formulated. The first is that SIP concentration, which is high under normal conditions until sexual maturation or shortly thereafter (32, 33), tends to remain high for some years subsequently unless depressed by gonadal secretions or sex hormones. Thus the SIP level tends (a) to remain elevated in subjects who fail to mature sexually (Tables 3 and 4) such as those with hypopituitarism, eunuchoidism (34, 35) or gonadal dysgenesis (36), and (b) to become elevated in mature persons whose gonadal secretions are eliminated by castration (present study) or reduced, as in some cases of Simmonds' disease (37) (Table 5) and of acromegaly (31, 38).

Some, although not all, of the differences among subjects in the degree to which gonadal secretions and sex hormones influence the SIP level may be associated with differences in age. SIP concentration is high in childhood (32, 33) but not in old age (39), although gonadal secretions are limited in both young and old. SIP concentration is not necessarily low in children with precocious development of secondary sex characters (Table 4), nor necessarily high in all adults in this[1] or other studies (2, 29) of subjects who lack secretions of testes or ovaries.[2]

[2] McCullagh and Renshaw (29) reported the SIP level to be 4.0 to 4.3 mg./100 ml. in 4 of 9 eunuchs, 33 to 65 years of age; the values in these 4 subjects (47 to 65 years age, mean of 54 years) were considered to be in the upper range of normal for adults. Values for the remaining 5 eunuchs (33 to 58 years of age, mean of 43 years) were 2.8 to 3.8 mg./100 ml. Apparently, only single determinations were reported for each man and it was not stated whether the blood was removed under basal conditions. Unfortu-

TABLE 5. SIP VALUES IN SUBJECTS WITH SIMMONDS' DISEASE, CHIEFLY AS COLLATED FROM THE LITERATURE AND PROVIDED BY DR. ROBERTO ESCAMILLA

The SIP level does not seem to be lower than in normal subjects of comparable age; in fact, it exceeded 4 mg./100 ml. in 3 of 6 adult patients for whom age, sex and values for SIP are recorded.

Patient		Serum P (mg./100 ml.)	Author, year, and reference in article by Escamilla and Lisser (*J. Clin. Endocrinol.* **2**: 65–96, 1942).
Age (yrs.)	Sex		
27	F	4.3	Barr and Mansbacher, 1936, #345.
31	F	2.6	De Langen, 1937, #280.
33	F	2.8	De Gennes, Delarue and Rogé, 1936, #392
48	F	4.2	Castleman and Hertz, 1939, #51.
48	M	4.4	Bulger and Barr, 1936, #269.
67	M	3.4	Boom, 1936, #201.
—	—	3.2	Meyler, 1938, #23.
—	M	3.6	Escamilla, personal communication.
—	F	4.7	Escamilla, personal communication.
—	F	3.1	Escamilla, personal communication.

The second concept is that high values for SIP as a result of reduced or absent gonadal secretions may tend to be lowered by gonadal secretions or stimulation by sex hormones. This phenomenon (a) is observed after sexual maturation under normal conditions (32, 33), and (b) may occur following administration of sex hormones in eunuchoidism (35), gonadal dysgenesis (35, 40, 41), and in some cases of acromegaly in which the SIP level has been high (31, 42).

Presumably, precocious development of secondary sex characters (Table 4) or intensive treatment of children with sex hormones may not necessarily lower SIP values to a great extent, at least for a while. Moreover, treatment with sex hormones would not be expected to decrease the SIP level to a marked degree in middle-aged or old subjects since, despite the reduction of steroid titers at these ages (3–10), untreated old persons do not exhibit the high SIP concentration that characterizes younger subjects with reduced gonadal secretions (39). Administration of sex hormones to postmenopausal women with osteoporosis (43) or to individuals with mammary or prostatic cancer (2) has resulted in only slight lowering of

nately measurements could not be obtained before orchiectomy. If the measurements are representative, they suggest a tendency to high SIP values in 44 per cent of the eunuchs, a tendency that is no less marked after 40 years of age than in the third decade of life.

SIP values.[3] Administration of stilbestrol induced significant retention of phosphorus in only 2 of 6 non-cancerous aged men as tested in balance studies; the 2 subjects who responded had osteoporosis (44).

Respective influences of sex and growth hormones. Until now, investigators (31, 42, 45) seem to have credited the endocrine regulation of SIP concentration largely to pituitary growth hormone. This assumption has enjoyed such general acceptance that in the absence as yet of adequate techniques for measurement of growth hormone, values for SIP have been used as a purported index of titers of growth hormone.

The present study and collated data suggest that in humans part of the regulation of SIP concentration, both under normal conditions and those of excessive growth, can be accounted for in terms of known differences in gonadal secretions and their demonstrated ability to depress the SIP level within the limits imposed by the body-wide metabolism of phosphorus. In this connection it should be noted that the SIP level is not high in all cases of definite acromegaly (38) and to the writers' knowledge seldom exceeds those observed in conditions, such as childhood or eunuchism, in which gonadal secretions are limited (46–48). Gonadal function is deficient in many acromegalics (49) and it may be that some acromegalics who have high titers of SIP have reduced amounts of circulating steroids.

A crucial test of the respective roles of sex and growth hormones is afforded by subjects with pituitary insufficiency in whom the secretion of both gonadal and growth hormones is presumably depressed. If growth hormone were solely responsible for elevated levels of SIP, hypopituitarism would be expected to result in markedly low SIP concentration. If sexual immaturity were a factor, however, SIP concentration would be expected to be normal in children and elevated in young adults. Hypopituitarism in young subjects with stunted growth and sexual immaturity (Tables 3 and 4) is associated with high levels of SIP, in keeping with the tendency to be expected in subjects with poor gonadal secretion. In an unstated number of subjects with chromophobe adenomas of the pituitary, Younghusband and associates (45) also found that SIP concentration was not low. The mean value was 3.8 mg./100 ml.; no statement was made as to the age of the subjects or whether the blood was withdrawn under fasting conditions. In 17 other subjects with chromophobe adenomas of the pituitary, most of whom were apparently in the fifth or later decades of life, the mean value for SIP (3.1 mg./100 ml.) was in the normal range (50). In this connection it may be recalled that in older subjects a decline in gonadal secretion is not accompanied by a high level of SIP (39).

Further information is provided by studies of subjects with gonadal dysgenesis, a condition in which epiphyseal closure is retarded and sexual maturation does not occur. In these subjects SIP concentration is said not to be depressed (51); when detailed data are provided (Table 4) SIP values tend to be in the upper normal adult range, or sufficiently high to be characteristic of the immature.

Finally it should be noted that administration of growth hormone preparations to primates did not increase SIP concentration either in intact or hypophysectomized

[3] Estrogenic treatment of patients with mammary cancer who showed no signs of metastases to bones induced only a 20 per cent decrease in SIP concentration; androgenic treatment was followed by a lowering of SIP concentration that was small, inconsistent, and of doubtful significance (2). Estrogenic treatment of men with prostatic cancer was associated with a tendency to reduction of the SIP level that was small, although consistent and sustained (2).

immature rhesus monkeys (52) or in human subjects (53). Insofar as these extracts represent active preparations, these findings would indicate that growth hormone does not elevate the SIP level in primates, even in those deficient in pituitary secretions. In dogs (54, 55) and rats (56), however, treatment with growth hormone increased the blood inorganic phosphorus level and prevented the decline that occurs in hypophysectomized rats. This suggests differences between species in the extent of the effect of growth hormone on SIP concentration. Species differ to a notable degree in their response to other types of pituitary secretions; for example, gonadotropic stimulation maintains spermatogenesis to a much better extent in rats than in primates (57, 58).

Thus, an inverse relationship between the SIP level and gonadal secretions should be recognized and the regulation of SIP concentration should not be attributed completely to supposed differences in titers of growth hormone which cannot be measured by procedures in current use. No inferences are intended, however, that growth hormone is without effect upon the SIP level[4] or that gonadal secretions are the sole regulating factor.

Other factors which influence the SIP level. Metabolism, as well as heredity, aging and other factors, probably determines the effect of gonadal secretions and steroids on the SIP level. The concentration of SIP is known to be altered by many conditions, especially those influential in intermediary metabolism of carbohydrates and those which affect bone metabolism or interfere with renal function (60). SIP values also tend to be high in hypoadrenocortical states induced by adrenalectomy or Addison's disease (61–63). SIP values were increased after adrenalectomy, even in juvenile monkeys, although admittedly these animals soon entered a terminal state (61). Thus, high values in hypoadrenalism and pituitary insufficiency may reflect depression of adrenocortical and other secretions, as well as those of the gonads.

Mode of action of sex hormones upon SIP concentration. There can be little doubt that sex hormones such as testosterone or estrogens reduce the urinary excretion of phosphorus in both immature (64) and mature subjects (34, 43, 65, 66). Conversely, elimination of gonadal secretions appears to enhance the tendency to osteoporosis after the menopause (43, 65) and in disuse atrophy of bone (67). It has been claimed that treatment with sex hormones (43, 67) counteracts the osteoporosis in these conditions.

Certain of the mechanisms through which sex hormones might conceivably affect SIP values seem unlikely in view of the present data. Elevation of the SIP level following castration is not to be credited to the enhancement of gonadotropic titers that may accompany castration. Values for SIP tend to be high in childhood and in subjects with pituitary insufficiency—conditions in which titers of gonadotropins are low.

The effects of gonadal secretions upon SIP concentration do not seem to be mediated through depression of parathyroid secretions, although it is true that in rats (68) and fowl (69) estrogenic treatment can reduce the rachitic effect of a diet deficient in vitamin D. Parathormone exerts a pronounced effect upon the level of serum calcium, whereas there is no change in serum calcium following castration.

Serum alkaline phosphatase was not studied in these subjects, but was found not to be altered in a consistent, sustained fashion following castration of 3 other eunuchs.[1]

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[4] In some acromegalics SIP values may exceed those attributable to deficiency of gonadal secretions. An example is the SIP value of 6.3 mg/100 ml. observed by Dr. Lawson Wilkins (59) in a 21 year-old acromegalic giant.

Values for the enzyme are stated to be normal in acromegalics treated with sex hormones (42). In rats (56), however, plasma alkaline phosphatase concentration is increased by treatment with growth hormone, even in hypophysectomized animals.

Proteins, hemoglobin and cholesterol. Castration did not alter blood protein values. A contrary finding has been reported in rats, in which castration increased the plasma globulin level (70). In humans the converse of castration, namely androgenic treatment, reduced excretion of urinary nitrogen, and of urea in particular (35), but also did not change levels of blood proteins (34, 71).

The decrease in hemoglobin and the increase in total cholesterol values were of such magnitude as to suggest the possibility of significant changes if larger numbers of subjects had been studied. Values for hemoglobin and erythrocytes in mature organisms tend to be higher in males than in females, and are known to be decreased by castration (72, 73).

Castration has been reported to elevate the total blood cholesterol level. McCullagh and Renshaw (29) observed values above 200 mg. per 100 ml. in 8 of 12 eunuchs. From 55 determinations in 40 subjects Teilum (74) concluded that values for cholesterol, although not augmented significantly during the first six months after castration, did become so during the next six months. Thereafter the increase was slow. Presumably the bulk of Teilum's subjects, like those in the present series, were in the age group in which cholesterol values increase materially with advancing years (75). Aging might have enhanced values after an elapse of four or more years but would not have had a significant influence upon Teilum's data in the first year after castration.

Teilum stated that the increase in serum cholesterol concentration tended to be especially marked in eunuchs who became fat but, unfortunately, Teilum used different subjects at different intervals of study. His statement is in keeping with the finding (76), however, that the level of serum cholesterol is correlated with body weight in men who are obese, but not in men of normal weight. In our series we observed no necessary relationship between the increases in cholesterol concentration and body weight after castration—but there were no major increments in body weight.

SUMMARY

The concentration of serum inorganic phosphorus (SIP) was studied under basal conditions before and after castration of 9 apparently healthy males, 18 to 38 years old. A statistically significant mean increase, to the range characteristic of children, developed within fifteen days after orchiectomy and was maintained throughout four years of subsequent study. The increase was greatest in subjects who had low values before castration. Substantial increases failed to occur in some subjects, however, especially in those whose values were high at the time of orchiectomy.

The definitive nature of the elevation in the SIP level after castration of young males with low SIP values (*i.e.*, in the adult range) is seen, in that orchiectomy did not induce sustained, statistically significant changes in the levels of serum calcium or other blood elements under study (sodium, potassium, uric acid, creatinine, glucose, pyruvic acid, lactic acid, cholesterol, lipid phosphorus, hemoglobin, total proteins, albumin, globulin, and cholinesterase).

Two concepts are advanced. The first is that SIP concentration (which is high until sexual maturation or shortly thereafter) tends (a) to remain elevated for several years subsequently in subjects who fail to mature sexually (eunuchoidism, hypopituitarism, and gonadal dysgenesis), and (b) to become elevated in mature persons whose gonadal secretions are eliminated by castration or reduced, as in Simmonds' disease and some cases of acromegaly.

The second concept is that high values for SIP, as a result of reduced or absent gonadal secretions, may tend to be lowered by gonadal secretions or by sex hormones. This phenomenon is observed after sexual maturation under normal conditions and may occur following administration of sex hormones in conditions with actual or presumed reduction of gonadal secretions (eunuchoidism, gonadal dysgenesis and acromegaly). Age seems to be one of the factors which influence the degree to which gonadal secretions and sex hormones affect the SIP level.

The effects of gonadal secretions on SIP concentration do not seem to be mediated by the secretion of the parathyroid glands or by gonadotropins.

Evidence is presented that the high SIP values in childhood, acromegaly and gigantism may be due in part to deficiency of gonadal secretions and not solely to postulated increases in titers of growth hormone.

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DECREASED ANTIDIURETIC RESPONSE TO BETA-HYPOPHAMINE IN HYPERTHYROIDISM*

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IN THE course of studies on the water retention induced by the administration of beta-hypophamine,[1] it was noted that a hyperthyroid subject failed to respond to 15 units of Pitressin-tannate-in-oil—a dosage much greater than that causing persistent antidiuresis in normal subjects (1, 2) and in cardiac patients (3). Because this observation supported the concept suggested by some investigators (4, 5), but questioned by others (6), that an antagonism exists between the effects of thyroid and posterior-pituitary hormones on water metabolism, the metabolic response to Pitressin was studied in 4 additional hyperthyroid subjects. The experimental findings indicate that hyperthyroid patients exhibit a greater resistance to Pitressin antidiuresis than can be explained on the basis of either increased total solute excretion or altered renal hemodynamics.

METHODS AND MATERIALS

The subjects of this study were 5 females with unequivocal clinical and laboratory evidence of severe hyperthyroidism. They were maintained under strict dietary control on a metabolic ward, and received a basic low-sodium (17–22 mEq.) diet and a constant measured water intake estimated to compensate for their increased losses by way of the skin and lungs. Three patients received additional sodium chloride (154 or 173 mEq. daily in 1-gram tablets) after meals.

The patients were weighed daily before breakfast on a beam balance accurate to 10 grams. Stools, pooled for six-day periods, and daily urines were refrigerated as collected, and analyzed for sodium, chloride, potassium, nitrogen, phosphorus and water. Urine total solute concentration (UTSC) was measured directly[2] in 3 subjects (PB, JL and DM) and calculated (from the urinary sodium, potassium and nitrogen concentrations)

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[1] Pitressin®—Parke, Davis & Co.

[2] By an osmometer manufactured by Fiske Associates, Boston, Mass.

in the others. Fasting venous blood was drawn without stasis at regular intervals and analyzed for serum and/or whole blood sodium, chloride, potassium, total protein, albumin, nonprotein and urea nitrogen, uric acid, creatinine, phosphorus, calcium, total solute concentration, and glucose. Hematocrits, eosinophil counts and daily urinary excretion of 17-OH-corticoids and creatinine were also determined. The methods used were those described in previous reports from this laboratory (7).

Following an adequate control period, the patients received increasing doses (2 to 15 units per day) of Pitressin-tannate-in-oil (P.T.O.) intramuscularly for a period of six to twelve days. The P.T.O. ampoules were warmed and well shaken to achieve homogeneity prior to administration. Injections were given at eight-hour to twelve-hour intervals rather than daily, in order to achieve a more constant sustained P.T.O. effect. After discontinuing hormone administration, the subjects were observed until their electrolyte balances and blood chemical values had returned to control levels.

Patient T.V. was given a second course of P.T.O. on the metabolic ward twenty-seven months after her initial hospitalization and successful treatment with radioactive iodine (I^{131}). Patient R.B.'s acute response to the intravenous injection of "physiologic" doses of aqueous Pitressin was also studied, both during her hyperthyroid phase, and again, one and a half years following I^{131} therapy, when she was clinically euthyroid.

To determine the influence of increased urinary total solute excretion on the response of a normal individual to P.T.O., a euthyroid man (E.M.) who previously had exhibited a typical normal response to Pitressin when on a low-sodium diet, was given P.T.O. while on an increased intake of sodium chloride and nitrogen as described subsequently.

RESULTS

Figure 1 presents the initial study on Patient T.V., whose metabolic diet was supplemented with 9 grams of sodium chloride. On a P.T.O. dosage of 5 units twice daily for six days and thrice daily for two additional days, negligible effects were demonstrable. During the control period, her daily urine output averaged 2,600 ml. and her calculated UTSC ranged from 292 to 325 mOsm/L. On P.T.O., her urinary output ranged from 3,225 ml. to 1,720 ml., the latter occurring only on the fifth day of the study; and the calculated urine osmolarity ranged from 309 to 377 mOsm/L. Her weight decreased 1.5 Kg. Concurrently, her serum sodium and chloride concentrations fell from 140 to 135 mEq./L, and from 102 to 98.4 mEq./L, respectively.

During the days when urinary sodium excretion increased, there was a slight increase in urine volume. After the P.T.O. injections were discontinued, a slight increase in urine volume and some sodium retention were observed, with a rapid rise in serum electrolytes to values above control levels.

When Patient T.V. was restudied twenty-seven months following I^{131} therapy, she was 4 Kg. heavier and was clinically euthyroid. At this time, she responded normally to 1 unit of P.T.O. twice daily with a significant fall in urine volume, a proportionate increase in UTSC and progressive gain in weight (Fig. 2). With the expansion of total body fluid volume,

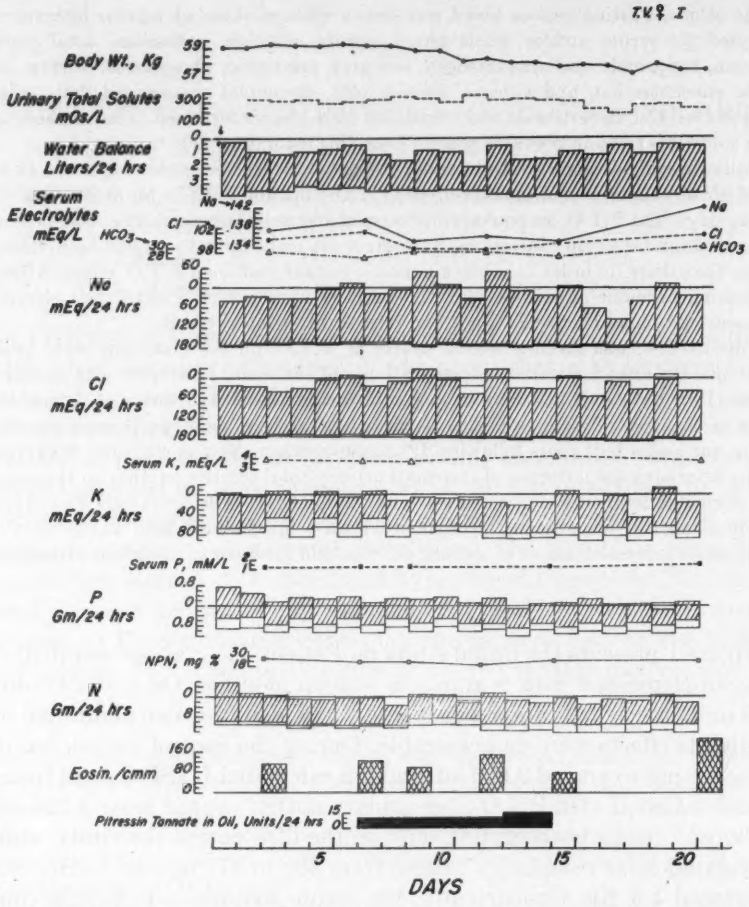

FIG. 1. Absence of antidiuretic response in Patient T.V., following large doses of P.T.O., when severely hyperthyroid.

In this and subsequent charts of metabolic data, outputs in the feces (open boxes), urine (cross-hatched boxes), vomitus (dotted boxes) and blood withdrawn (black boxes) are superimposed on the intakes, which are plotted downward from the zero or base line.

the urinary electrolyte excretion increased despite the continued fall in serum electrolyte concentration. As a consequence of the resulting osmotic diuresis, the UTSC failed to rise, although the daily P.T.O. dose was increased to 3 units on the third day and to 4 units on the fourth.

On the fourth day, recurrent vomiting and increasing natriuresis led to a negative sodium balance of 84 mEq. and a weight loss of 0.3 Kg. On the

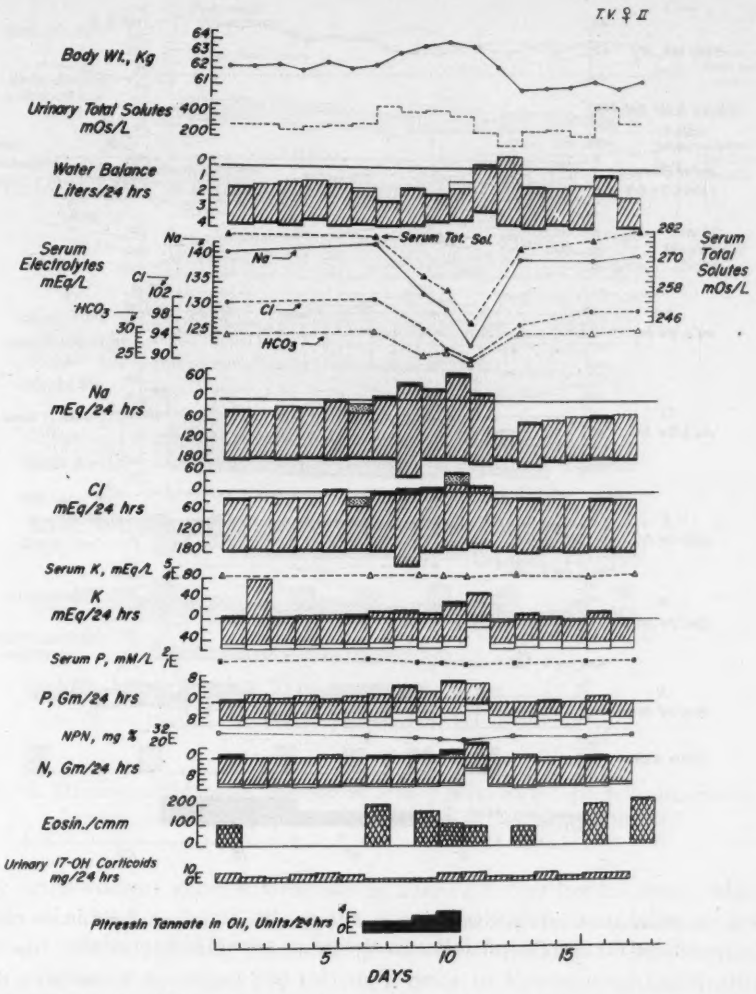

FIG. 2. Normal antidiuretic response of Patient T.V., following small doses of P.T.O., when euthyroid twenty-seven months after I^{131} therapy. On days 8 and 11, because of nausea and vomiting, 1 liter of isotonic sodium chloride solution intravenously was substituted for the oral salt tablets.

fifth day, the serum sodium and total solute concentrations were 123 mEq./L and 245 mOsm/L, respectively. Following discontinuance of P.T.O., water diuresis ensued with a corresponding fall in UTSC. Following the loss of 2.8 Kg. in body weight, sodium and chloride balances be-

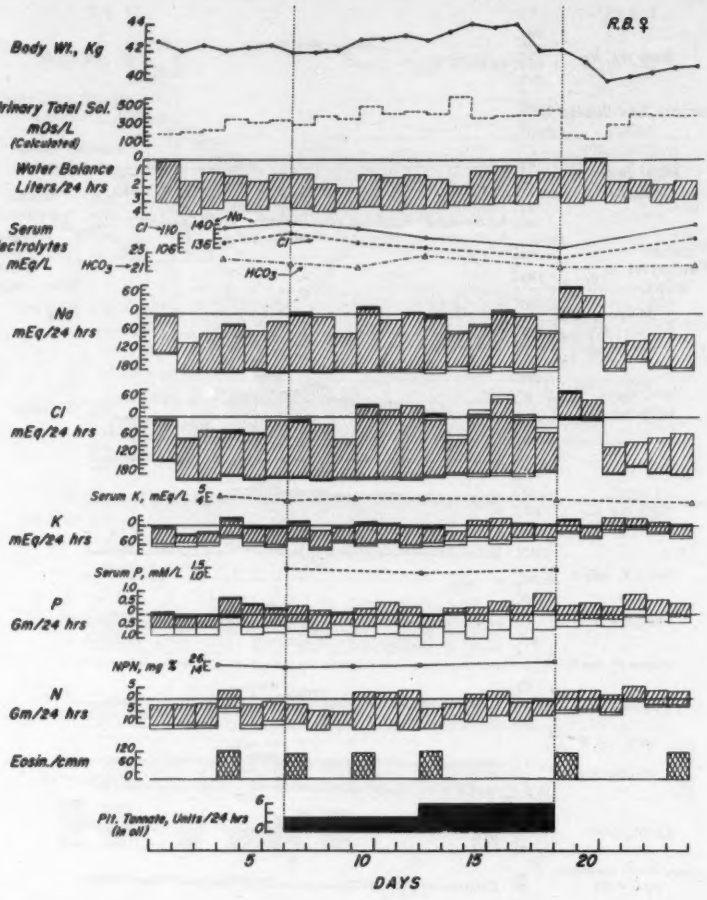

FIG. 3. Minimal antidiuretic response to 3 to 6 units of P.T.O. daily in severely hyperthyroid Patient R.B., maintained on normal sodium intake.

came positive and serum electrolyte and total solute concentrations returned to virtually normal levels by the end of the study. At this point, fluid intake was reduced by 2,000 ml. for one day, to test the patient's response to endogenous antidiuretic hormone. Her urine volume fell promptly, with a rise in urinary total solute concentration equivalent to that obtained during the period of P.T.O. administration.

Figure 3 presents the data on Patient R.B., who also received supplementary salt tablets. When given first 1 unit and, later, 2 units of P.T.O. three times daily for six days, she exhibited only a minimal response, her

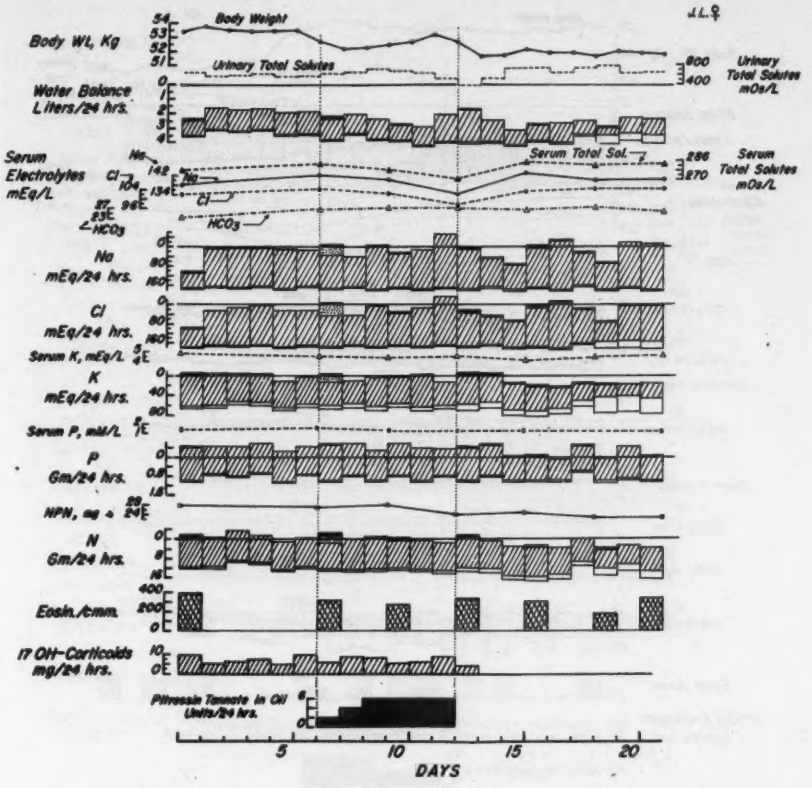

FIG. 4. Minimal antidiuretic response to 2 to 6 units of P.T.O. daily in severely hyperthyroid Patient J.L., maintained on normal sodium intake.

daily urine volume generally exceeding 2,000 ml. Her lowest urine volume of 850 ml., on the second day of the 6-unit daily dose, was followed by a 2,000-ml. diuresis during the ensuing twenty-four hours. The calculated urine osmolarity averaged 300 mOsm/L prior to Pitressin administration, and increased to above 500 mOsm/L on only two days while the hormone was given. A marked drop in urinary total solute concentration was noted during the two days following cessation of the drug. This was due not only to Pitressin withdrawal, but also to a decreased urinary sodium excretion following the inadvertent omission of the sodium chloride tablets. During this study, body weight changes were small and serum sodium and chloride levels fell only 4 mEq./L.

Figure 4 illustrates the study on Patient J.L., who was given supplementary sodium chloride tablets. She exhibited a minimal antidiuretic

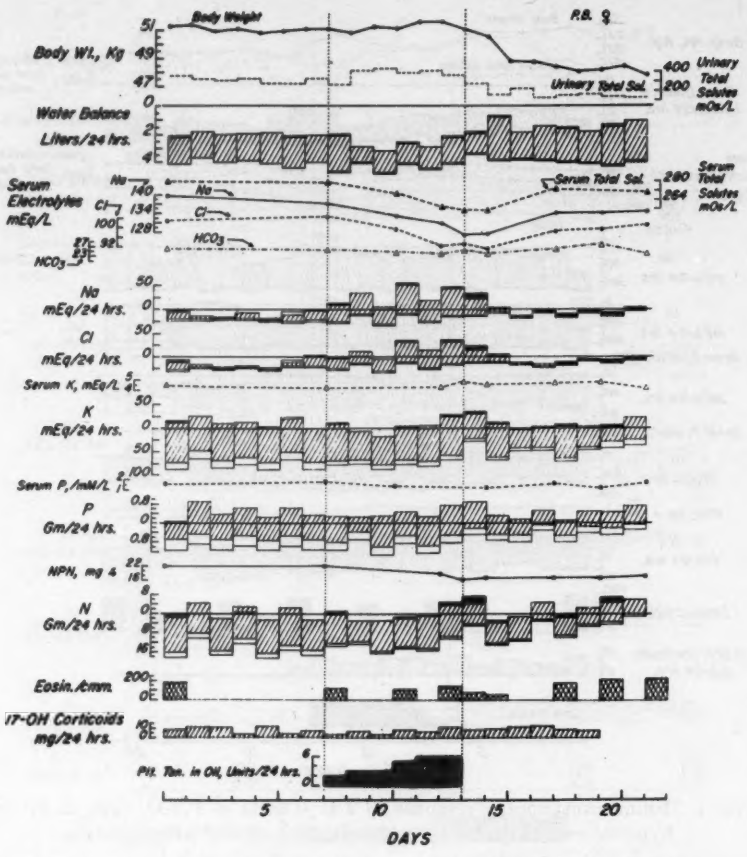

FIG. 5. Moderate antidiuretic response to 2 to 6 units of P.T.O. daily in hyperthyroid Patient P.B., maintained on low-sodium intake.

response to 1 to 3 units of P.T.O. twice daily. While on P.T.O., her daily urine osmolarities varied from 501 to 708 mOsm/L, as compared to the control values of 562 to 640 mOsm/L. During the day following discontinuance of Pitressin, her urine volume rose to 2,215 ml. with an osmolarity of only 392 mOsm/L but without any change in total solute excretion, and her weight fell 1 Kg.

Serum electrolytes reflected only mild dilution, and rapidly returned to control levels after the hormone was discontinued. As in the other patients, no changes were observed in eosinophil counts or balances of potassium, nitrogen or phosphorus.

In Figure 5 are presented the data on Patient P.B. In order to evaluate

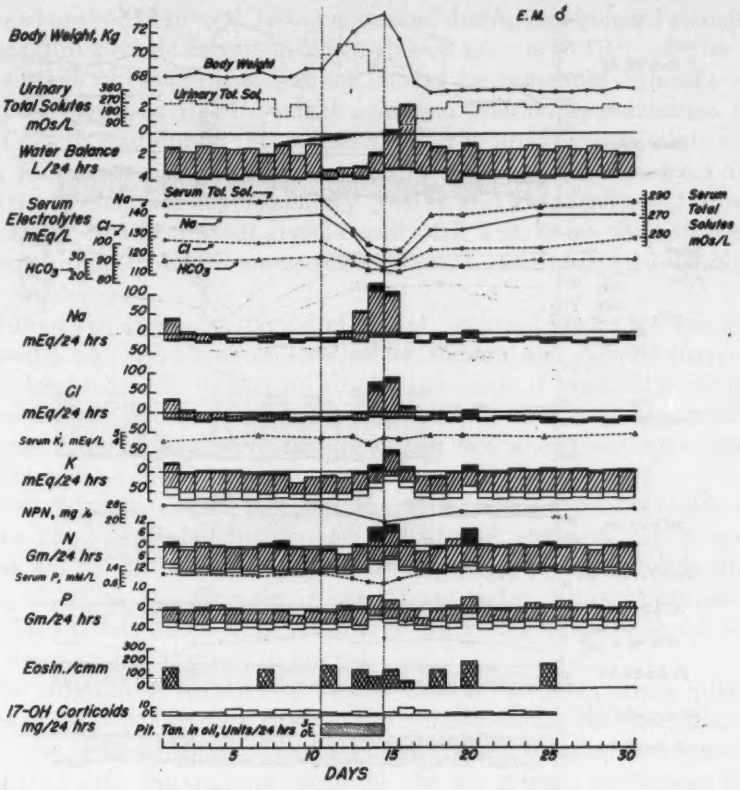

FIG. 6. Typical antidiuretic response to 3 units of P.T.O. daily in normal Subject E.M., when on low-sodium intake.

the role of the urinary solute load in the diminished response of the hyperthyroid patient to P.T.O., no supplementary salt tablets were given to P.B. and to a fifth hyperthyroid patient (D.M.) who exhibited a similar response. During the control period, weight remained fairly constant, and daily urine volumes and UTSC averaged 2,000 ml. and 320 mOsm/L, respectively. During the first day, when she was given 1 unit of P.T.O. at twelve-hour intervals, the urine volume increased to 2,580 ml. and UTSC fell to 251 mOsm/L. On the second and third days, she received 1 unit of P.T.O. three times and her urine output fell to 1,135 ml., with an increase in UTSC to 419 mOsm/L. During the next three days, when she was given either 5 or 6 units of P.T.O. per day, the urine volumes remained under 2,000 ml. and UTSC averaged 400 mOsm/L. By the sixth day, the serum sodium, chloride and total solute concentrations had fallen to 127 mEq./L,

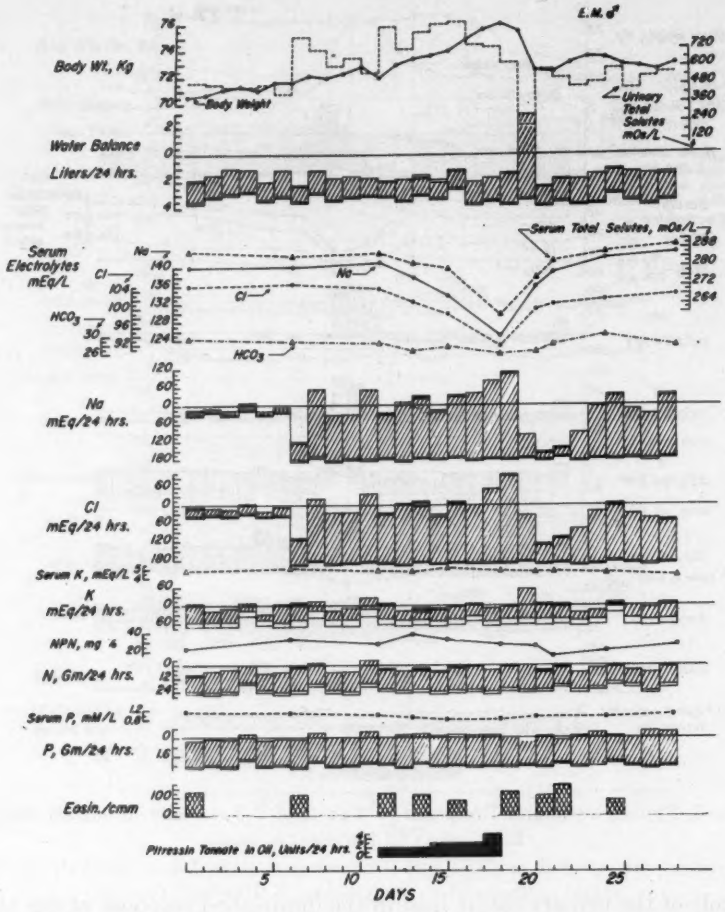

FIG. 7. Typical antidiuretic response of Patient E.M. to 2 and 3 units of P.T.O. daily, despite the increased urinary total solute excretion induced by markedly increased intakes of salt and nitrogen.

93.7 mEq./L, and 254 mOsm/L, respectively. Despite her probable caloric deficit, she had a net weight gain of 0.5 Kg. during this period.

During the first forty-eight hours after the P.T.O. was discontinued, the urine output was 4,680 ml. and body weight fell 2.3 Kg. Following this water diuresis, serum electrolyte and total solute concentrations rose promptly but failed to reach control levels, in spite of virtually complete retention of the small amounts of sodium and chloride in the diet.

Figures 6 and 7 present observations on Patient E.M., the *euthyroid*

man whose response to P.T.O. was studied during diet-induced changes in urinary total solute excretion. When given 3 units of P.T.O. daily for four days while on a 25-mEq. sodium intake, he responded typically with oliguria, increased UTSC, weight gain, and dilution hyponatremia (Fig. 6). Prior to, and during this second study, the urinary total solute excretion was increased to 573 mOsm/day by giving him a dialyzed milk-protein hydrolysate supplementary feeding and an additional 154 mEq. of sodium chloride. When given 1 unit of P.T.O. twice daily, the urine volume dropped, UTSC rose to 885 mOsm/L, and he gained 0.9 Kg. in one day.

During the seven-day period of P.T.O. administration, his weight increased 4 Kg., and his serum total solute, sodium, and chloride concentrations gradually fell to 260 mOsm/L, 125 mEq./L and 92.3 mEq./L, respectively. The 24-hour urine volumes remained low, the maximal volume (1,750 ml.) occurring on the seventh day, when there was a marked natriuresis and chloriuresis.

After discontinuing the hormone, the urine volume increased to 2,315 ml. in the first twenty-four hours, and to 5,630 ml. the next day, following which the serum sodium level rose to 136 mEq./L, and chloride to 100 mEq./L. With the decrease in total body water, urinary sodium and chloride concentrations dropped to very low values until the serum levels and body weight returned to normal.

The antidiuretic response of Patient R.B. to the intravenous injection of small doses of Pitressin (Fig. 8) was studied during her first admission when she was severely hyperthyroid, and eighteen months after successful treatment with I^{131}. On both occasions, she was given a continuous intravenous infusion of 5 per cent glucose in water at a rate of 16.5 ml./min. When a constant maximal urine flow was achieved, a dose of 5 m.u. (milliunits) of Pitressin was injected intravenously. After the return of urine output to control values a dose of 24.3 m.u. of Pitressin (0.57 m.u./Kg. of initial body weight) was injected into the tubing.

When hyperthyroid, she had no antidiuresis following the 5-m.u. dose of Pitressin (Fig. 8). However, shortly thereafter she became somewhat restless and showed a slight decrease in the flow of urine. Following the larger dose, the urine flow fell promptly from a control value of 16.6 ml./min. to a minimal value of 6.0 ml./min. during the second ten-minute period, rose quickly to the control value fifteen minutes later, and subsequently reached a peak of 19 ml./min.

By the time of the second acute study, the patient was unequivocally euthyroid and had gained 9.6 Kg. in weight. Now, the administration of 5 m.u. of aqueous Pitressin caused a drop in urine volume from 14 ml./min. to 7.4 ml./min., with a return to control values within forty to fifty min-

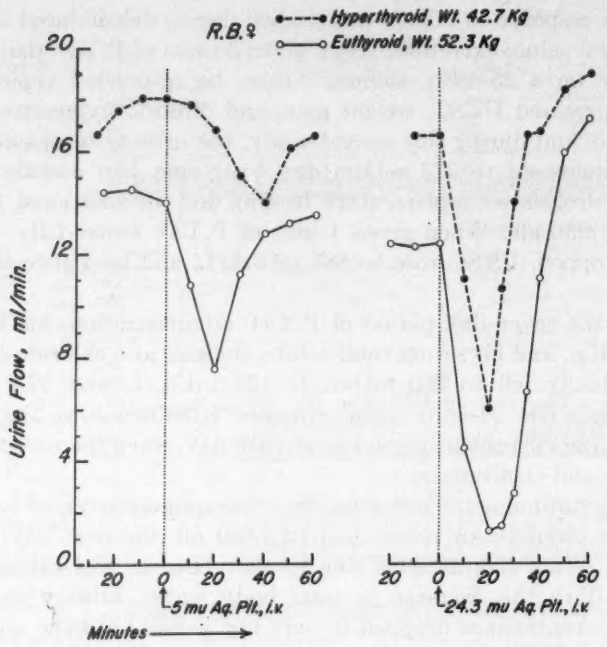

FIG. 8. Diminished response of Patient R.S. to intravenously injected physiologic doses of aqueous Pitressin when severely hyperthyroid (closed circles and broken lines), in contrast to when euthyroid (open circles and solid lines) eighteen months following I^{131} therapy.

utes. Following administration of 24.3 m.u. of Pitressin, which was only 0.46 m.u. per Kg. of body weight at this time, the urine volume fell from 12.4 ml./min. to a minimum of 1.2 ml./min. during the second ten-minute period, and did not reach the control value until forty-five minutes after the injections.

During the second study in this patient, the urinary sodium excretion rate was consistently higher than during the first study; in spite of the greater total solute loads, the urine volumes were consistently lower than previously both before and after Pitressin, under a comparable water load. Her inulin and PAH clearances at the time of the second study were normal, being 119 and 675 ml./min./1.73 sq.M., respectively.

DISCUSSION

Although the 5 hyperthyroid subjects ultimately received doses of P.T.O. three to seven times greater than those given in previous studies to either our cardiac patients (3) or normal subjects (1, 2), none exhibited

comparable antidiuretic responses. In general, the changes in urinary volume and total solute concentration, and extracellular electrolyte concentrations were negligible in 1 and minimal in 2 of the patients on the higher sodium intakes, and were considerably reduced in the 2 patients on the low-sodium intake. Since all 5 were actively hyperthyroid, definite weight loss would normally be anticipated on their 2,400–3,000 calorie intakes. However, even after correcting for their estimated caloric deficit and measured negative nitrogen balances, their weight gains from water retention while receiving Pitressin were minimal, despite the increased fluid intakes given to compensate for their greater insensible water loss.

The relationship between the effects of the thyroid and the posterior-pituitary antidiuretic hormones on water metabolism has been previously studied in patients and animals with diabetes insipidus. It is well known that frequently the polyuria is partially ameliorated by thyroidectomy, only to return when thyroid hormone is administered (4–6, 8–13). However, there has not been complete agreement as to the mechanisms involved.

Some have attributed the diuretic effect of thyroid in these experiments to the general increase in body metabolism, but such metabolic stimulants as dinitrophenol have failed to induce similar results (9, 13). Consequently, it has been suggested that this may represent a specific function of thyroid hormone.

In 1935, Pitts (14) showed that the urea and xylose clearances were elevated in dogs following either ingestion of meat or administration of thyroxine. He suggested that in both cases enhanced metabolism of protein, whether exogenous or endogenous, might be the cause of the altered renal hemodynamics. Hare *et al.* (5) reported that administration of dried thyroid gland to dogs with diabetes insipidus induced both an increase in glomerular filtration rate and a decrease in the renal tubular reabsorption of water following the intravenous administration of physiologic doses of Pitressin. They suggested that the observed changes in water reabsorption resulted from a direct antagonism between the two hormones. Leaf *et al.* (15) showed that in a patient with combined anterior and posterior pituitary deficiency, maintained on a constant urinary excretory load, increasing the BMR by administration of thyroxine caused no increase in urine volume or decrease in urinary total solute concentration. Their conclusion that the diuretic effect of thyroid results solely from the increased urinary solute load is consistent with the earlier suggestion of Swann and Penner (6) that urinary total solute load, and particularly sodium excretion, are important determinants of urine volume in rats with diabetes insipidus, both before and after thyroidectomy or thyroid administration.

The present studies suggest that the diminished antidiuretic response of

severely hyperthyroid patients to beta-hypophamine is largely independent of the increased urinary solute excretion resulting from their hypermetabolism. That increased urinary solute load and excessive insensible water loss in sweat might ordinarily contribute some apparent resistance to this exogenous antidiuretic material is undeniable. However, their water intakes were increased sufficiently to compensate for the hyperhidrosis. Moreover, in a normal subject, even larger total urinary solute loads, produced by markedly increased dietary intakes of salt and nitrogen, failed to prevent the typical Pitressin-induced water retention and increased urinary total solute concentration (Fig. 7).

The mechanism of this seeming antagonism between thyroid and posterior pituitary antidiuretic material remains to be established. An increased rate of inactivation of beta-hypophamine as a consequence of the increased metabolism of the hyperthyroid state is one possibility. Unless inactivation of beta-hypophamine occurs almost instantaneously, such acceleration could not explain the complete absence of response of Patient R.B. to 5 m.u. of Pitressin when hyperthyroid. However, her lesser antidiuresis and her more rapid recovery following the 24.3-m.u. dose, like the diminished response in the hyperthyroid dog with severe diabetes insipidus (5), could result from such a mechanism.

Various clinical observations also suggest that there may be some antagonism between the effects of thyroid and posterior pituitary hormones on water metabolism. In 1937, Findley (16) observed clinical improvement and increased responsiveness to Pitressin following surgical total thyroidectomy in 1 case of diabetes insipidus. Rieber and Silver (17), in 1952, reviewed the clinical literature and reported amelioration of the symptoms of both hyperthyroidism and long-standing diabetes insipidus in another patient following successful treatment with radioactive iodine. More recently, Crispell, Parson and Sprinkle (18) found that patients with untreated primary myxedema exhibited a poor diuretic response to oral or intravenous water loads. This abnormality of water metabolism was unaffected by doses of cortisone which overcome a similar deficiency in Addisonian subjects, but was completely corrected when the euthyroid state was achieved by administration of thyroid.

The present observations do not permit analysis of the suggested role of augmented renal hemodynamics in the diuresis induced by thyroid administration. However, very marked increases in renal hemodynamics would be required to explain the diminished antidiuretic response to beta-hypophamine in severely hyperthyroid patients. Moreover, the available literature and the present limited studies do not reveal sufficient changes in the glomerular filtration rate or renal plasma flow in hyperthyroidism to influence water excretion.

The more normal responses to chronic Pitressin-tannate-in-oil injections and to acute intravenous injections of Pitressin in Patients T.V. and R B., respectively, after successful treatment of their hyperthyroidism, suggest that the present observations do not represent chance variation, despite the small series of hyperthyroid patients. Furthermore, preliminary studies indicate that an increased resistance to the antidiuretic effects of P.T.O. develops in normal subjects rendered hyperthyroid by large doses of triiodothyronine.

It remains to be established whether this increased resistance of the hyperthyroid subject to beta-hypophamine represents increased destruction of this hormone, or some decreased sensitivity of the renal tubules as a consequence of a diuretic effect of thyroid hormone. However, the failure of thyroidectomy or thyroid administration to influence water excretion *per se* in severe diabetes insipidus, when presumably no posterior pituitary antidiuretic hormone (ADH) is present (5), indicates that thyroid hormone itself is not diuretic but acts only to counteract the influence of ADH. This concept is supported also by the observation that normal water balance ordinarily is preserved in hyperthyroid patients or animals, despite increased insensible loss and augmented urinary solute excretion. With their intact hypothalamic-posterior pituitary regulatory mechanisms, an increased rate of release of ADH would result from any acceleration of inactivation, for example. That the thyroid may influence the rate of inactivation of circulating ADH is suggested by the delayed and impaired diuretic response to water in myxedematous subjects (18), whose filtration rates may be no lower than those of patients with moderately severe congestive failure, who respond normally to intravenous hydration (19). In such hypothyroid patients, although a normal osmoreceptor response to decreased extracellular tonicity probably results in diminished ADH release, water diuresis may be delayed because of a slower rate of disappearance of previously circulating antidiuretic hormone.

SUMMARY

1. Three severely hyperthyroid patients on regular sodium intakes, in contrast to normal subjects and cardiac patients, when given 2 to 15 units of beta-hypophamine in the form of Pitressin-tannate-in-oil (P.T.O.) daily for six to twelve days, failed to achieve the anticipated antidiuresis and increase in urinary total solute concentration. Two hyperthyroid patients on low-sodium diets also exhibited diminished responsiveness to P.T.O. Following successful treatment with I^{131}, 1 patient showed a normal antidiuretic response to P.T.O.

2. In 1 of these hyperthyroid patients, the intravenous administration of physiologic doses of aqueous Pitressin also failed to induce acute anti-

diuresis comparable to that observed subsequently when she was euthyroid following I^{131} therapy.

3. That increased urinary total solute loads alone can not explain the apparent resistance of hyperthyroid patients to beta-hypophamine is suggested by the observation that a normal subject whose urinary solute excretion was markedly augmented by increased oral intakes of salt and nitrogen responded normally to P.T.O.

4. Possible mechanisms for this apparent refractoriness of hyperthyroid subjects to beta-hypophamine are discussed.

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THE ACTION OF MONO-AMMONIUM GLYCYRRHIZINATE ON ADRENALECTOMIZED SUBJECTS AND ITS SYNERGISM WITH HYDROCORTISONE*

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MOLHUYSEN *et al.* (1) demonstrated that licorice extract taken orally by human subjects acted on the electrolyte balance in a manner similar to that of desoxycorticosterone acetate, namely, with the retention of sodium and the excretion of potassium. Groen and his associates (2, 3, 4) extended these findings to demonstrate that glycyrrhizinic acid[1] and its hydrolysis product, glycyrrhetic acid obtained from licorice, were the active ingredients of the extract. Though Groen *et al.* had some success in the maintenance of patients with Addison's disease on licorice preparations, Borst and his associates (5) noted that these preparations in a case of Addison's were ineffective when used alone. However, they did note that small doses of cortisone used in conjunction with licorice were quite effective in maintaining the electrolyte balance in adrenal deficiency states, and suggested that those cases of Addison's disease which did respond to licorice still had remnants of functional adrenocortical tissue (5). Recently Hudson *et al.* reported some studies on the use of small doses of cortisone with licorice preparations in adrenalectomized patients (6, 7).

This study[2] comprises data on the effectiveness of mono-ammonium glycyrrhizinate (MAG) in the maintenance of 2 adrenalectomized patients. Clinical and metabolic observations were made during a period in which varying doses of MAG and hydrocortisone were administered. Laboratory observations included determinations of serum potassium, serum sodium and plasma cholesterol levels, blood eosinophil counts, and urinary sodium, potassium and 17-ketosteroid excretion.

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[1] Glycyrrhizinic acid consists of a disaccharide moiety and one molecule of the triterpine, glycyrrhetinic acid.

[2] The authors are deeply indebted to Dr. Lincoln Lebeaux for his invaluable assistance in the execution of this study.

Mono-ammonium glycyrrhizinate was kindly supplied by Dr. C. K. Swift of MacAndrews Forbes Company.

ADRENALECTOMIZED SUBJECTS

The subjects selected for this study were 2 chronic schizophrenic female patients (C. P. and P.M.), in each of whom the psychosis was of fifteen years' duration. Both patients had had carcinoma of the breast for which radical mastectomy had been performed prior to their admission to Worcester State Hospital. Bilateral adrenalectomy was performed on June 16, 1952. Although both patients were said to have metastasis, evidence of metastasis was observed only in Patient C.P., who prior to adrenalectomy had an indurated immobile mass the size of a lemon in the postoperative mastectomy scar on the left. Following the adrenalectomy, the mass gradually decreased in size, and by the time of this study was no longer palpable.

In preparation for the mono-ammonium glycyrrhizinate (MAG) experiment, the daily oral dose of 80 mg. of hydrocortisone which the patients were receiving while other studies were in progress, was gradually reduced over a period of sixteen days to 40 mg. per day, at which time the MAG was started in a dosage of 4.0 Gm. by mouth daily with 2.0 Gm. of sodium chloride. *The sodium chloride (2.0 Gm. per day) was given throughout the entire study.* Prior to the beginning of the experiment, both patients were alert, active and voiced no somatic complaints. Both patients had voracious appetities and slept well. During the sixteen-day period immediately prior to the administration of MAG, the blood pressure of Patient C.P. ranged from 144/70 to 130/80, and that of Patient P.M. varied between 104/56 and 102/70.

CASE HISTORIES

Subject C.P.

On May 4, 1953, MAG was started in a dosage of 4.0 Gm. a day orally, in conjunction with oral hydrocortisone, 40 mg. daily. The patient was kept on this regimen for seven days. The medication schedule may be seen in Figure 1. During this period no changes were observed, except a tendency to sleep more. On May 11, the hydrocortisone was reduced to 20 mg. a day. During this period the patient's appetite, though adequate, was not voracious as it had been and she was less active. On May 13, pitting peripheral edema was noted for the first time and this persisted for three days before subsiding. During one period when MAG was reduced from 4.0 to 2.0 Gm. daily (May 18–22), the patient's appetite became poor, she was lethargic and exertional dyspnea was noted for the first time. When MAG was increased to 4.0 Gm. a day, the patient showed marked clinical improvement and despite the fact that on May 25 the hydrocortisone was reduced to 10 mg. per day, her condition remained satisfactory for the next eleven days. The lethargy disappeared and her food intake returned to its previous ravenous level. The only abnormality on physical examination during this period was an occasional extra cardiac systole.

On June 8, hydrocortisone was discontinued and the patient was maintained on MAG, 4.0 Gm. per day. For the first twenty-four hours, no untoward symptoms were

noted. However, by the second day she lost all spontaneous volubility, answered questions with a paucity of words and spoke only in monosyllables. The tendency to sleep became prominent and the patient remained in bed all day. Peripheral edema returned and the blood pressure dropped to 122/72, from the 140/90 observed on June 8. On June 11, the patient was lethargic, weak, anorexic and took small amounts of liquid only after strong urging. There was a marked oliguria and she passed three watery stools during the day. The blood pressure dropped to 114/89, the temperature rose to 100.6° F rectally and the pulse was rapid at an average rate of 104 per minute. Since the patient's condition was becoming worse and circulatory embarrassment was evident, hydrocortisone was resumed in a dosage of 10 mg. per day orally. On June 12, a clysis of 1,000 cc. of 5 per cent glucose was given. No improvement was evident for the next thirty-six hours. However, by the evening of the second day after resumption of hydrocortisone, definite improvement was discernible and by the third day the patient was eating well, spoke spontaneously and got out of bed. By June 17, the peripheral edema had disappeared and no signs of circulatory embarrassment were evident.

On June 29, hydrocortisone was again discontinued and the patient was maintained on MAG, 4.0 Gm. per day. The clinical status was characterized by increasing irritability, restlessness, lethargy, lack of appetite, weakness and somnolence—a clinical picture which was similar to that which occurred during the previous period when the hydrocortisone was discontinued. On July 4, oral hydrocortisone in a dosage of 20 mg. per day was begun. The patient's condition was again markedly improved. Gradually the hydrocortisone was increased to 40 mg. a day and on July 15 the MAG was discontinued. The clinical status of the patient was satisfactory during the remainder of the study.

Subject P.M.

In Patient P.M. the medication schedule was similar to that in Patient C.P. On May 4, 1953, MAG was started in a dosage of 4.0 Gm. daily, the oral dose of hydrocortisone having been gradually decreased over the preceding sixteen days from 80 mg. per day to 40 mg. per day. The following day the patient became restless, paced the floor and although she frequently returned to bed, did not sleep soundly. The next day her face was swollen and an erythematous flush of butterfly configuration covered the nose and the malar prominences. This condition was diagnosed as erysipelas. Although the patient's temperature was not elevated, she was given penicillin, 300,000 units a day, for three days. By May 8, the swelling and discoloration of the face had subsided. She recovered from this facial infection with no signs of adrenal deficiency; there was no increase in the daily dose of hydrocortisone. When the dosage of hydrocortisone was reduced to 20 mg. a day on May 11, pitting peripheral edema developed; she complained of fatigue and was quite lethargic. These untoward manifestations persisted for two days and then spontaneously disappeared without change in the medication schedule. On May 18, the hydrocortisone was decreased to 10 mg. and the MAG to 2.0 Gm. daily. Despite the development of a florid perineal cellulitis which required 600,000 units of penicillin for relief, the patient's general physical condition remained satisfactory and there were no signs of adrenal embarrassment. On May 22 MAG was increased to 4.0 Gm. daily.

On June 8, the hydrocortisone was discontinued, but the MAG was kept at 4.0 Gm. per day. At 6:00 A.M. the following day the patient complained of nausea and vomited a small amount of greenish liquid. The temperature was 100.4° F and the pulse was weak and rapid at 114 per minute. The blood pressure was stable at 120/80. The following day

the temperature rose to 102° F. Because of the hyperpyrexia, persistent nausea and vomiting, tenderness to palpation in the right upper quadrant and the history of a previous attack of empyema of the gallbladder, a diagnosis of acute cholecystitis was made. On June 10, penicillin was started in a dosage of 600,000 units twice a day, and parenteral fluids were given.

On June 12, the patient had a brief period of unconsciousness during which she slumped in the nurse's arms while attempting to use the bed pan. The blood pressure dropped to 100/80, and the possibility of impending adrenal crisis prompted the resumption of hydrocortisone, 10 mg. per day. The patient responded well to hydrocortisone at this dosage level and a satisfactory clinical status was restored on June 14. Throughout the next two weeks her condition was good, with the maintenance of normal eating and sleeping habits.

On June 29, hydrocortisone was again discontinued. On July 1, the patient was observed to be drowsy. Drowsiness continued during the following day, but physical examination revealed no other abnormality. On July 3, she was lethargic, complained of nausea, and refused to eat dinner or supper. During the night she retained only small amounts of fluid. However, no circulatory embarrassment was evident and the blood pressure was 142/80. On July 4, hydrocortisone was resumed in a dosage of 20 mg. a day orally. The patient remained listless and nauseated, but did not vomit. On July 7, she had pain in her left shoulder and vomited periodically throughout the day. She complained of feeling cold and her skin was cold to touch, although her temperature was 99.8° F rectally. Her blood pressure dropped to 90/52, the pulse was feeble and rapid, and circulatory embarrassment was evident. The inability of the patient to retain oral medication necessitated the intravenous administration of hydrocortisone, 100 mg. in 500 cc. of saline. Though a favorable clinical response to this measure was promptly discernible, it was of short duration. The following day she again lapsed into a state of crisis. She was given hydrocortisone, 100 mg. in 500 cc. of saline intravenously, and cortisone acetate, 25 mg. three times a day intramuscularly. She responded favorably to these measures and by the following evening her clinical status had significantly improved. This improvement was maintained. On July 12, the cortisone acetate was reduced to 25 mg. per day intramuscularly and on July 16 it was discontinued. On July 17 the MAG was discontinued and the patient remained in good clinical condition for the remainder of the study on an oral maintenance dosage of 40 mg. of hydrocortisone daily.

Both of these patients had had schizophrenia for many years, for which they had been institutionalized. At the time this study was instituted both patients showed the cardinal signs of this illness, *i.e.*, a flat, inappropriate affective response, disorganization of thought processes, tangential and irrelevant associations, restriction of the scope of awareness and a complete loss of insight. The psychiatric status of both patients remained unchanged throughout the entire period of study, except on those occasions when they lapsed into an adrenal crisis. On such occasions both patients showed none of the cardinal signs of schizophrenia on examination. However, as soon as adrenal balance was restored by appropriate treatment the unmistakable signs of an active schizophrenic process again became evident in both subjects.

METABOLIC STUDIES

Blood

Figures 1 and 2 show the blood pressure, eosinophil counts, serum Na/K ratio, plasma cholesterol level, and the medication schedule of MAG and adrenal steroids for C.P. and P.M., respectively.

In general the eosinophil pattern was similar in both patients. During the first period of hydrocortisone discontinuance an abrupt increase in the count was noted. With return to a dosage of 10 mg. of hydrocortisone a day, the count became stabilized at the higher levels. Again, with the second

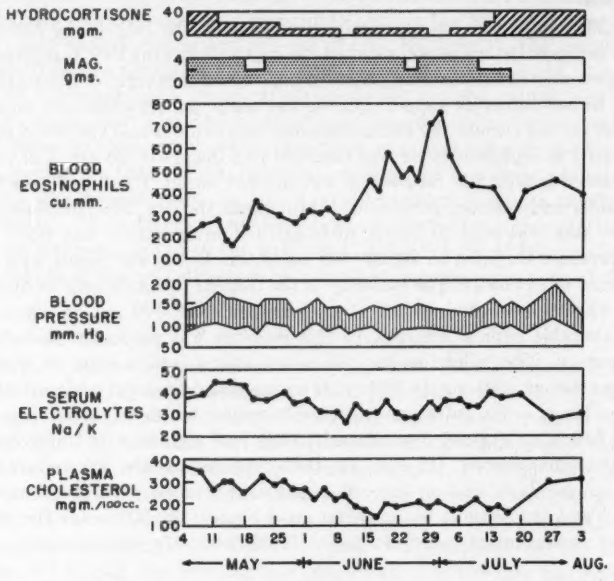

FIG. 1. Medication schedule and blood data on adrenalectomized Patient C.P.

period of steroid abstinence the eosinophil count rose promptly. It may be observed that Patient P.M. showed a pronounced rise in the eosinophil count during the adrenal crisis; following the intravenous administration of 100 mg. of hydrocortisone there was a marked drop. By the end of the study period the eosinophil counts had decreased steadily. However, both patients at this time showed higher eosinophil counts than at the initiation of the observations.

There is evidence to suggest that the serum Na/K (molar ratio) and blood pressure are positively related. Increases and decreases of the Na/K ratio followed very closely the corresponding changes in blood pressure. In the course of the study it became evident that these fluctuations were also

related to the clinical condition of the patients (*see* case histories). The blood pressure and electrolyte ratio at the end of the study, when a dose of only 40 mg. of hydrocortisone was being administered, were lower than when 40 mg. of hydrocortisone was supplemented by 4.0 Gm. of MAG at the beginning of the observation period.

A feature of the blood chemistry data which was not related to the clinical condition of the patients but rather to the dose of hydrocortisone, was the plasma cholesterol level. In both patients a steady decline in the

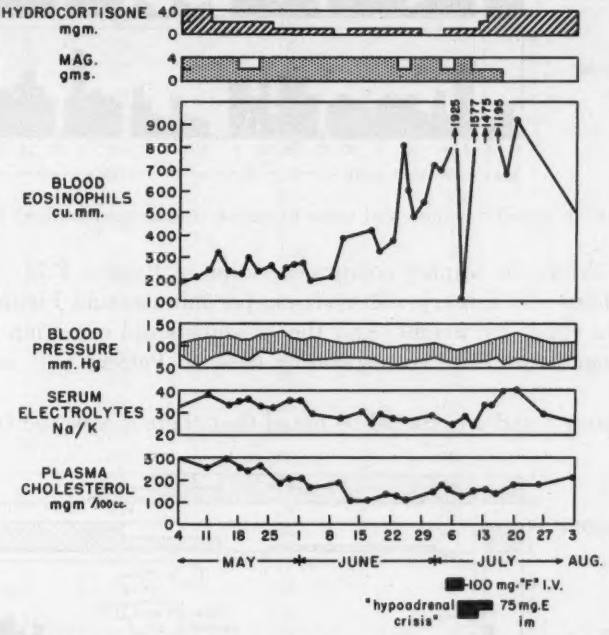

FIG. 2. Medication schedule and blood data on adrenalectomized Patient P.M.

cholesterol values was associated with a reduction in the dose of hydrocortisone. The lowest cholesterol values were observed during the periods when the patients received no hydrocortisone or when the dosage was reduced to 10 mg. a day. With the return to higher dosage levels of hydrocortisone there occurred a steady rise in the cholesterol values to those observed at the initiation of the study.

Urine

Urine collections were made on both patients during the study. Patient P.M. was more cooperative, and therefore her data are more complete.

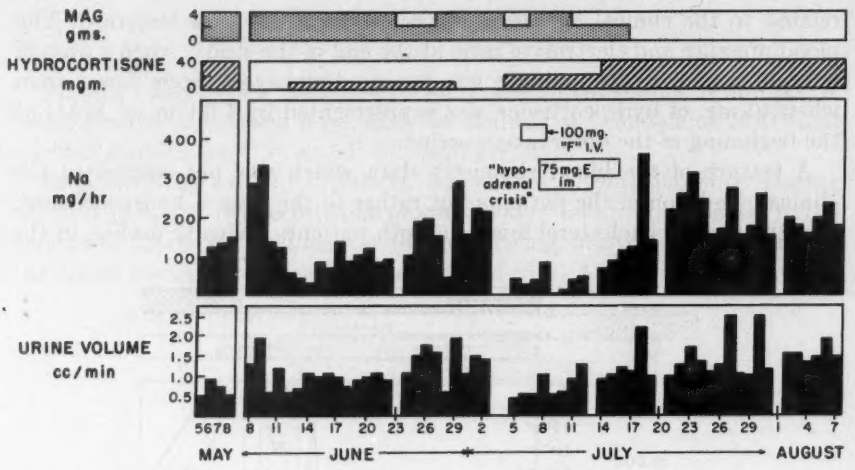

FIG. 3. Rate of urinary sodium and urine excretion in adrenalectomized Patient P.M.

Figure 3 shows the urinary sodium excretion of Patient P.M. recorded in mg. per hour and urinary volume in cc. per minute; and Figure 4 depicts the K/Na ratio (by weight) and the 17-ketosteroid excretion in mg. per twenty-four hours. The corresponding data on Patient C.P. are listed in Table 1.

In Figures 3 and 4 it should be noted that there is a period from May 8

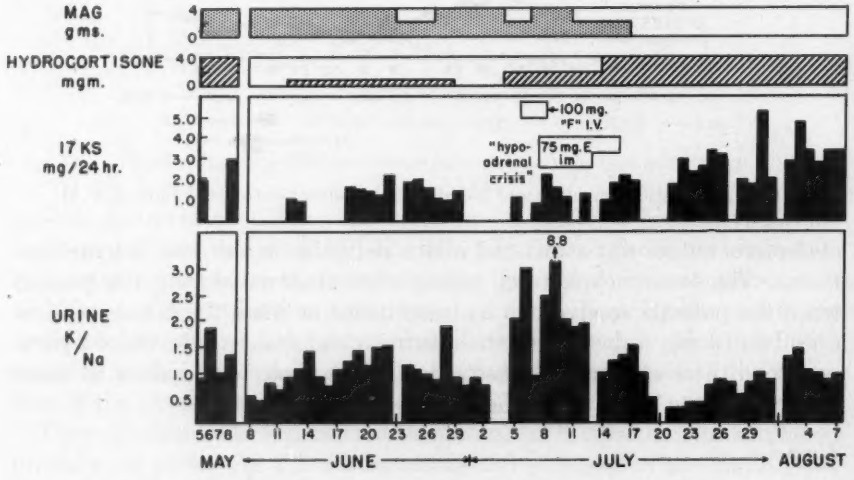

FIG. 4. 17-Ketosteroid excretion and K/Na ratio of urine in adrenalectomized Patient P.M.

TABLE 1. URINARY ELECTROLYTE AND 17-KETOSTEROID EXCRETION OF PATIENT C.P.*

Date (1953)	Urine vol. (cc./min.)	Na (mg./hr.)	K (mg./hr.)	17-KS (24 hrs.)
May 5	.49	120	101	4.32
6	.85	142	153	—
7	.60	115	94	—
8	.69	131	118	2.40
June 8	.49	103	131	—
9	.48	63	35	—
14	.67	18	14	.60
15	.47	33	17	—
16	.52	69	47	—
19	.34	36	48	.62
24	.79	74	41	.70
26	.61	91	36	.53
27	.40	62	37	.72
28	.53	43	26	.72
29	.31	31	44	.76
30	.35	34	26	—
July 7	.43	23	160?	1.82
8	.38	46	53	.87
9	.86	86	103	.98
10	.53	35	30	.65
11	.67	75	99	.86
12	.80	95	110	1.18
14	.81	78	92	1.10
15	.74	90	61	1.20
17	.80	112	72	1.68
18	.50	68	49	1.61
21	.36	74	34	1.20
22	.55	85	45	1.08
24	.80	136	61	1.56
25	.72	212	143	2.38
29	.58	85	102	1.99
30	.74	—	—	2.40
Aug. 4	.76	142	107	2.88
5	1.10	169	111	3.12
6	.94	150	113	2.40
7	.93	164	124	1.80

* *See* Figure 1 for medication schedule, and text for case history.

to June 8 when no collections were obtained.

During the period when Patient P.M. was receiving 40 mg. of hydrocortisone daily supplemented by 4.0 Gm. of MAG, the 17-ketosteroid excretion rate was 2–3 mg. per twenty-four hours. Immediately following the first period, during which hydrocortisone was withheld, the 17-ketosteroid excretion decreased to less than 1 mg. per day. At a dosage level of 10 mg. of hydrocortisone daily the steroid excretion rose slightly, varying between 1 and 2 mg. per day. Again, during the second period of hydrocortisone abstinence beginning on May 29, the steroid excretion dropped to less than 1 mg. per day. With the resumption of larger doses of hydrocortisone an increase in 17-ketosteroid excretion was noted. At the end of the study the excretion values had returned to the levels of 2–3 mg. per day and higher which prevailed at the initiation of the study. It should be noted that during the period of administration of intramuscular cortisone acetate and intravenous hydrocortisone, the increase in 17-ketosteroid excretion was not commensurate with the amount of steroids administered.

The volume of urine excreted appeared to be correlated with the amount of sodium excreted. This was strikingly evident in Patient P.M. (Fig. 3). During the first period in which hydrocortisone was withheld there was a pronounced loss of water and sodium, which was followed by a reduced urinary volume and sodium excretion. During the period of marked clinical deterioration (June 10 through June 11) this relationship was also reflected by a decline in the urinary K/Na ratio (*cf.* Fig. 4). With the resumption of hydrocortisone in a dosage of 10 mg. per day, the sodium excretion slowly increased and continued at the levels which existed a month earlier during the period when the patient was receiving hydrocortisone, 40 mg. daily supplemented by 4 Gm. of MAG. On May 23, when the MAG was reduced to 2 Gm. per day, an increase in sodium excretion was again noted, but this was not as large as during the omission of hydrocortisone. Upon the resumption of MAG (4.0 Gm. a day) on May 27, marked sodium retention was evident by the second day. These changes in electrolyte balance were reflected by decreases and increases in the urinary K/Na ratio. When the patient was maintained on 4.0 Gm. of MAG per day in the absence of hydrocortisone, inability of the patient to retain sodium was again observed. Upon resumption of hydrocortisone (20 mg. per day), the electrolyte pattern returned to normal, showing sodium retention and an increase in the K/Na ratio. However, when the patient was unable to retain oral medication, adrenal crisis occurred. The measures taken to control the adrenal crisis, namely, the administration of hydrocortisone intravenously and cortisone acetate intramuscularly, caused further reten-

tion of sodium and excretion of potassium as indicated by the high urinary K/Na ratio. When the MAG was discontinued on July 17, there was a marked increase in the excretion of sodium, which remained high for the remainder of the study except for a slight decrease observed by the end of the period of observation.

Figure 5 contains data regarding the sodium excretion and urinary K/Na ratio of Patient P.M. during the period in which hydrocortisone (40 mg. per day) was being administered. Placebos of lactose were given during the control period and during the experimental period when the

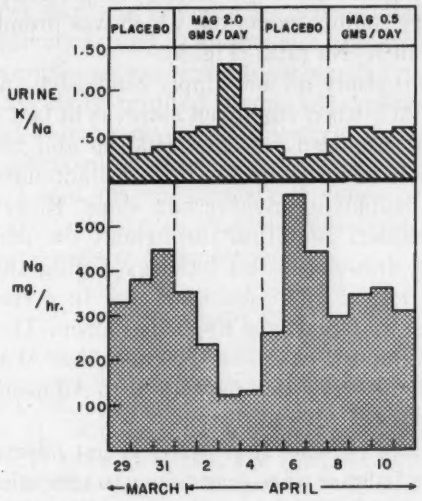

FIG. 5. The effect of varying doses of MAG on the electrolyte excretion of Patient P.M. maintained on 40 mg. of hydrocortisone and 2.0 Gm. of sodium chloride orally per day.

patient was receiving MAG, 2.0 and 0.5 Gm. per day respectively. It may be seen that a daily oral dose of 2 Gm. of MAG has a potent sodium-retaining effect when supplemented by 40 mg. of hydrocortisone, but not when employed in conjunction with a daily dose of only 10 mg. of hydrocortisone (*cf.* Fig. 3). The smaller dose of 0.5 Gm. of MAG has little if any sodium-retaining effect.

DISCUSSION

MAG alone, given in the dosage employed in these studies, was ineffective in the maintenance of 2 adrenalectomized patients. On two occasions an attempt was made to maintain the patients on 4.0 Gm. of MAG per day alone (2.0 Gm. of salt per day was given throughout the study).

Within the first twenty-four hours there was a loss of sodium and fluid, followed promptly by a decline in the clinical status of both patients which was evident by the second day. However, as little as 10 mg. of hydrocortisone orally in conjunction with the 4.0 Gm. of MAG per day was effective in maintaining a satisfactory clinical state, as well as fluid and electrolyte balance. On the occasions when the dose of MAG was reduced from 4.0 to 2.0 Gm. daily while hydrocortisone was maintained at a level of 10 mg. daily, there was a perceptible decline in the clinical status associated with sodium and fluid depletion. When Patient P.M. was given 2.0 Gm. of MAG while receiving 40 mg. of hydrocortisone per day, there was a sharp reduction in urinary sodium excretion, which was promptly reflected by a corresponding rise in K/Na ratio (Fig. 5).

The results of this study do not supply conclusive answers to the question of whether MAG, when employed alone, is in fact sufficient to maintain life in adrenalectomized patients. Hudson and his associates (6, 7) apparently were able to maintain life in 1 of 3 adrenalectomized patients for several weeks, employing glycyrrhizin alone. However, all 3 patients were in a grave clinical condition throughout the period of treatment. The occurrence of drowsiness and lethargy during the abstinence from hydrocortisone is not, in all probability, due to a specific MAG effect, but rather to the state of extreme hypoadrenalism. These studies confirm the observation of Borst *et al.* (5) to the effect that MAG alone is ineffective in the maintenance of certain patients with Addison's disease in whom there are no remnants of adrenal tissue.

The metabolic data indicate that MAG is not effective in the maintenance of electrolyte balance when given alone to adrenalectomized patients. The electrolyte data on both serum (Figs. 1 and 2) and urine (Figs. 3 and 4) also show this to be the case. However, in conjunction with small doses of hydrocortisone, MAG in a daily dosage of 4.0 Gm. is quite potent. Furthermore, MAG had no effect on the eosinophil count or the plasma cholesterol level. The steady decline in the cholesterol values was closely related to the hydrocortisone dosage. The possibility of diet playing a role in determining the plasma cholesterol level must be considered. However, it is apparent in both patients that, though a period of loss of appetite occurred, this was only short lived and on the occasions when the patients were receiving 10 mg. of hydrocortisone in conjunction with 4.0 Gm. of MAG per day, their appetites were as voracious as at any time throughout the entire period of study. It is probable that these observations on cholesterol values are similar to those of Di Luzio *et al.* (8), who showed that plasma lipid levels were increased when adrenalectomized dogs were given large doses of cortisone in addition to desoxycorticosterone acetate.

SUMMARY

Two adrenalectomized patients who were given mono-ammonium glycyrrhizinate (MAG) in an oral dosage of 4 Gm. daily were not maintained in a satisfactory clinical state.

The desoxycorticosterone acetate-like action of MAG, observed in subjects with intact adrenals, was absent.

When as little as 10 mg. of hydrocortisone per day was given orally with 4.0 Gm. of MAG, both adrenalectomized patients were maintained in excellent clinical condition. Reduction of the dosage of MAG from 4.0 Gm. to 2.0 Gm. per day was followed by a deterioration in the clinical condition of the patients as well as an increase in sodium excretion. When the dosage of hydrocortisone was raised to 40 mg. daily, the administration of 2.0 Gm. of MAG daily resulted in marked sodium retention. Thus, hydrocortisone and mono-ammonium glycyrrhizinate have synergistic effects in the adrenalectomized patient.

Eosinophil counts and plasma cholesterol values were unaffected by mono-ammonium glycyrrhizinate. Plasma cholesterol values were positively related to the dosage of hydrocortisone.

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STUDIES OF THE ADRENAL HYPERFUNCTION IN 2 PATIENTS WITH ATYPICAL CUSHING'S SYNDROME

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THE variation in both the clinical and biochemical response to administered adrenal steroids or corticotropin suggests that naturally occurring adrenal hypercorticism might not always be manifested as fully developed Cushing's syndrome. Until recently, the diagnosis of adrenal hyperfunction depended for the most part on typical clinical findings, since analytical methods for the corticosteroids were not quantitatively reliable. Within the past few years newer methods of steroid analysis have been developed which permit a more accurate measure of cortical function. One of these procedures (1, 2) has been used extensively in the course of evaluating many patients with one or more of the features of Cushing's syndrome. Among these were patients with obesity, hirsutism, diabetes, osteoporosis or hypertension. The 2 patients who are the subject of this report were found to have cortical hyperfunction. Their appearance so belied Cushing's syndrome that the correct diagnosis had not been made by many competent observers, even after prolonged study. The predominant feature was the extreme wasting of bone, muscle and skin, and in one patient this advanced atrophy represented the end-result of continued adrenal hypercorticism of thirty years' duration. In this clinical report we will refer to our observations of the adrenal defect in 6 additional patients with Cushing's syndrome (3). The endocrine data from these studies will be presented in a later report.

CASE REPORTS

Case I

M.M. was a 41-year-old Indiana millhand. He was first seen in the Henry Ford Hospital Orthopedic Division in October 1953, with a chief complaint of pains in his back and ribs for the previous fifteen months. During this time he had lost 3 inches in height and 17 pounds of weight. He complained of moderate weakness and a tendency to bruise easily; large ecchymoses frequently developed following moderate trauma. Bone pains and fractures led him to seek help in several clinics and in July 1953 a diagnosis of von Recklinghausen's disease of bone was made. However, exploration of the neck failed

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to demonstrate any abnormal parathyroid tissue. Studies carried out in other clinics were said to demonstrate marked bony abnormalities but no diagnosis was made.

Systematic review of the history yielded little additional information. The patient denied headache or visual disturbances. He had no gastro-intestinal or genito-urinary complaints. His libido and potency remained normal. A chronic dermatomycosis of the feet and right hand had been present for many years. He had always been of ruddy complexion and no striking change in his appearance had been noted, other than his stooped posture. His skin had become thinner and was more shiny than usual, and he had experienced chronic ankle edema for over a year without any cardiorespiratory symptoms.

FIG. 1. Case I, M.M.

Examination revealed a kyphotic, red-faced, middle-aged white man weighing 65 Kg. (Fig. 1). There was neither "moon" face nor "buffalo hump." The extremities were slender and somewhat wasted; the thick stocky appearance of the trunk was due to the bowed dorsal spine and a barrel-shaped chest. The skin was atrophic and ecchymoses developed readily at the site of needle punctures. Visual fields were intact. The blood pressure averaged 140/80; the size and auscultatory findings of the heart were normal. Although the antero-posterior diameter of the chest was increased, the breath sounds were normal. There was no abnormality of the abdomen, rectum or external genitalia. Skeletal roentgenograms were interpreted to show advanced decalcification of the ribs, spine and skull, with multiple rib fractures and compression fractures of three thoracic vertebrae (Fig. 2). Extraosseus calcification was seen in the area of both supraspinatis tendons and adjoining several of the interphalangeal joints. Fragments of bone were present in the right elbow joint. The sella turcica was normal. Intravenous pyelograms were normal.

The following pertinent laboratory data were obtained initially: hemoglobin, 14.4 Gm. per cent; red blood cell count, 4.5 million; hematocrit, 55 per cent; white blood cell count, 9,800 with 64 per cent polymorphonuclear leukocytes and 36 per cent lymphocytes, the latter being predominantly leukocytoid. There was diminished cellularity of the marrow, an increased percentage of normoblasts, and no myeloma cells. The level of serum nonprotein nitrogen was 31 mg. per 100 ml., and of fasting blood sugar, 106 mg. per 100 ml. The urine was free of sugar, but contained albumin on occasion. Bence-Jones protein was not present. The serum electrolyte levels were as follows: sodium, 138 mEq./L; potassium, 5.4 mEq./L; CO_2, 25.6 mEq./L; and chloride, 97 mEq./L. Results of the intravenous glucose tolerance test were normal. Using 35 grams of glucose, with blood specimens obtained at 0, 15, 45, 75, and 150 minutes, the blood sugar values were 103, 177, 146, 109 and 85 mg. per 100 ml. The serum albumin level was 4.0 Gm. and the globulin 1.6 Gm. per cent. The direct eosinophil count at 3 P.M. varied from 114 to 154 cells per cu. mm. The concentration of serum calcium was

10.1 and 10.3 mg. per 100 ml.; phosphorus, 2.2 and 2.0 mg. per 100 ml.; alkaline phosphatase, 3.4 and 2.9 Bodansky units; and acid phosphatase, 1.0 King-Armstrong unit.

Continuous collections of urine, from 7 A.M. to 7 A.M., were started shortly after the patient's admission to the hospital and he was given first a creatine-free and creatinine-free diet, and then a 150-mg. calcium diet. Urine specimens for steroid studies were refrigerated without preservative during the day of collection and then frozen until the day of analysis. 17-Hydroxycorticoids in urine were determined by a modification (1) of the Reddy procedure (2). 17-Ketosteroids in urine were determined by the Klendshoj modification (4) of the Drekter procedure (5). Creatinine in urine was determined by the method of Folin (6).

Spontaneous creatinuria varied between 60 and 170 mg. and creatinine excretion averaged 1,380 mg. per twenty-four hours. While receiving the low-calcium diet, he excreted 120 mg. and 134 mg. of calcium in two consecutive 24-hour urine specimens.

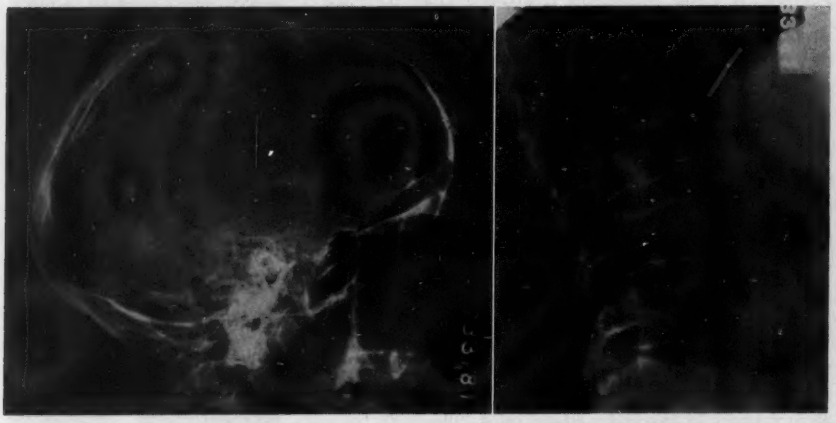


FIG. 2. Roentgenograms of the skull and spine in Case I, showing advanced demineralization and compression fractures.

17-Hydroxycorticosteroid excretion was markedly elevated and averaged 25.7 mg. per twenty-four hours. The average 17-ketosteroid excretion of 24.7 mg. was in the high normal range. Gonadotropins were present in the urine in amounts greater than 8 and less than 16 mouse units per twenty-four hours.

After several days of observations on basal steroid excretion, the patient was given 10 clinical units of corticotropin-gel twice daily for two days. This resulted in a much greater than normal increase in urinary 17-hydroxycorticoids (Table 1).

These findings were interpreted to indicate that adrenocortical hyperfunction was the probable cause of the marked bone atrophy, and exploration of the suprarenal areas was carried out on November 10, 1953. As both adrenal glands appeared normal (Fig. 3), a simultaneous bilateral total adrenalectomy was performed. The patient received 100 mg. of cortisone intramuscularly the night before and the morning of operation. The postoperative course was complicated by a right pleuritis with effusion, but this subsided promptly with antibiotic therapy. The dosage of cortisone was progressively reduced from 200 mg. to 50 mg. daily by the tenth postoperative day. In addition, the patient received daily 2.5 mg. of desoxycorticosterone in oil. He was discharged from the

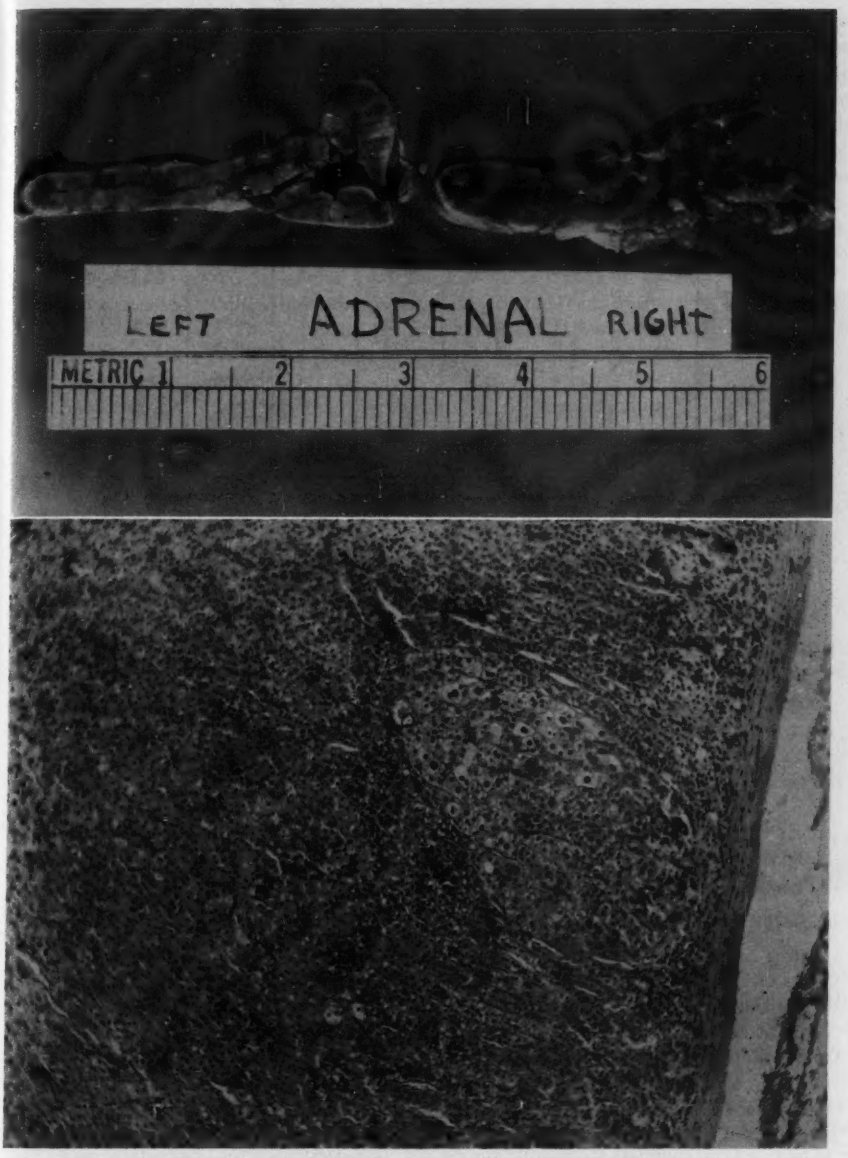


FIG. 3. Case I. The right adrenal weighed 6 Gm. It was fragmented on surgical removal. The left adrenal weighed 9 Gm. Both glands showed similar histologic structure with focal areas of hyperplasia in the cortex. The hyperplastic cells were three to five times normal size, with foamy cytoplasm and large hyperchromic, bizarre nuclei. The remaining cells were somewhat atrophic and showed cytoplasmic lipoidal depletion. (H and E ×105.)

TABLE 1. URINARY HORMONE EXCRETION IN THE 2 PATIENTS WITH ADRENOCORTICAL HYPERPLASIA COMPARED TO THAT IN NORMAL SUBJECTS

	Urinary 17-hydroxycorticoids				Calculated endogenous ACTH†	17-Ketosteroids		Gonadotropin, m.u.u./24 hrs.
	Control		During ACTH*			Control	During ACTH	
	mg./24 hrs.	mg./Gm. creatinine	mg./24 hrs.	mg./Gm. creatinine		mg./24 hrs.	mg./24 hrs.	
CASE I (M.M.)	25.7	18.2	67.5	41.2	6.5	24.7	43.9	>8<16
NORMAL MALE								
Range	7–15.5	5–12	10–37	7–33	2–13	8–32	10–38	8–64
Average	11.5	8.0	18.9	18.3	6.7	16.8	20.5	16
CASE II (W.Y.)‡	9.9	12.8	29.8	45.1	1.9	12.9	18.3	0
NORMAL FEMALE								
Range	5–9	4–8	17–25	13–23	1.6–4.9	8–19	14–28	4–32
Average	7.5	6.3	21.4	17.3	3.4	12.8	21.2	8

* Dosage 10 clinical units of corticotropin-gel subcutaneously every 12 hours.

† Clinical units of ACTH necessary to obtain the control level of urinary 17-hydroxycorticoids calculated from the response to exogenous ACTH.

‡ This patient received only 5 units of ACTH every 12 hours.

hospital on the fifteenth postoperative day. During convalescence, severe aching and stiffness of the joints developed; this was most prominent in the shoulders and right elbow, which had been shown to be abnormal by x-ray examination prior to operation. These symptoms gradually subsided with increased dosage of cortisone. Weakness, marked cold sensitivity and anorexia were prominent for the next several months, and 50 mg. of cortisone daily was required to maintain well-being. During the first postoperative month the fungus dermatitis became generalized, but it responded promptly to treatment.

Gradually the patient gained in weight and strength. His bone pains and bruising subsided, the ankle edema disappeared and the skin texture returned to normal. The blood pressure remained normal. The patient was able to return to his former occupation four months after operation.

Case II

W.Y., a 48-year-old cashier, first had persistent ankle edema at the age of 17, two years after the menarche. At this time there was also swelling of the face and easy bruising of the skin. Several "nervous breakdowns" had occurred in girlhood. An appendectomy at the age of 21 was uneventful, except that the return of menses was delayed for many months. Although normally active, she was handicapped by nervousness, persistent edema, increasing fatigue and weakness. In 1930 she consulted a surgeon with these complaints. Although the basal metabolic rate was said to have been −17 per cent, and the thyroid apparently was not enlarged, a subtotal thyroidectomy was performed. This led to an increase in the weakness and fatigue, and the menses ceased entirely, not to return for the next eight years. Desiccated thyroid in large amounts did not relieve her symptoms, and the edema continued unabated.

In 1933 the patient entered the Toledo Hospital for studies, the results of which have been made available through the courtesy of Dr. Glenn H. Reams, of Toledo,

Ohio. Examination at that time revealed a very dry skin and edema of the lower extremities, which were discolored a deep red to purple. The face was flushed and swollen. The BMR was −37 per cent. The bleeding and clotting times were normal. The level of serum calcium was 10.9 mg. per 100 ml.; phosphorus, 4.2 mg. per 100 ml.; fasting (blood) sugar, 94 mg. per 100 ml.; nonprotein nitrogen, 30 mg. per 100 ml.; and chloride, 88 mEq./L. A diagnosis of myxedema was made, and therapy with desiccated thyroid was continued. The symptoms of edema, discolored skin, bruises, weakness, and amenorrhea persisted. The BMR ranged from −14 to −31 per cent during therapy.

In 1934 the patient first entered the Henry Ford Hospital for examination, at which time the physical findings were much the same. She weighed 123 pounds and her blood pressure was 160/108. Additional laboratory study of the blood revealed a carbon dioxide combining power of 28.3 mEq./L, a serum albumin level of 4.8 Gm. per cent, and globulin, 1.8 Gm. per cent. There was a trace of albumin in the urine, and phenolsulfonphthalein excretion was normal.

FIG. 4. Case II, W.Y.

Subsequently, while she was under the care of Dr. Reams, a skull roentgenogram was interpreted to show a possible suprasellar tumor. She re-entered the Toledo Hospital in 1936 for further evaluation. In the three-year interval between hospital admissions she had gained 10 pounds and now presented a plethoric adiposity of the face, thorax and abdomen. There was no abnormal hair. The upper extremities were thin. The legs, still edematous, were bronze in color and exquisitely painful to pressure. Scars on the legs were the sites of previous spontaneous ruptures of the skin from tension of the underlying edema. There were long purple striae over the buttocks. The blood pressure was 200/120. Skull x-ray examination was reported to show "erosion of the sella turcica." With this evidence, a diagnosis of Cushing's disease was made and an unknown amount of roentgen therapy was directed to the pituitary in April 1936. No discernible improvement was noted after two months.

She was admitted to the Toledo Hospital for the third time in July 1937, essentially unchanged. After a prolonged interval with no improvement, she underwent exploration and biopsy of the left adrenal gland. It was reported to be entirely normal (Fig. 6A). She left the hospital the following January with less edema but otherwise not improved. However, regular menses returned spontaneously a short time later.

In August 1944, the patient returned to the Henry Ford Hospital as an employee. Except for her now regular menses, she had not changed significantly since the studies ten years before. The edema, bruises, weakness, hypometabolism and mild hypertension slowly but relentlessly progressed. In 1946 she fell, incurring a laceration of the right knee which remained unhealed for a month. It finally closed under a thin atrophic scar. By 1949 her weight had fallen to 121 pounds. She reported frequent headaches and spontaneous conjunctival hemorrhages. Further x-ray examinations of the skull were reported to show osteoporosis but no abnormality of the sella turcica. The skin and muscle wasting became so profound that the patient entered the hospital in 1950 for further studies. The hemoglobin was 15.6 Gm. per cent and the hematocrit 51 per cent. The white blood cell count was 5,800 with 76 per cent granulocytes and 24 per cent lymphocytes. The direct eosinophil count was 13 cells per cu. mm. The serum albumin level was

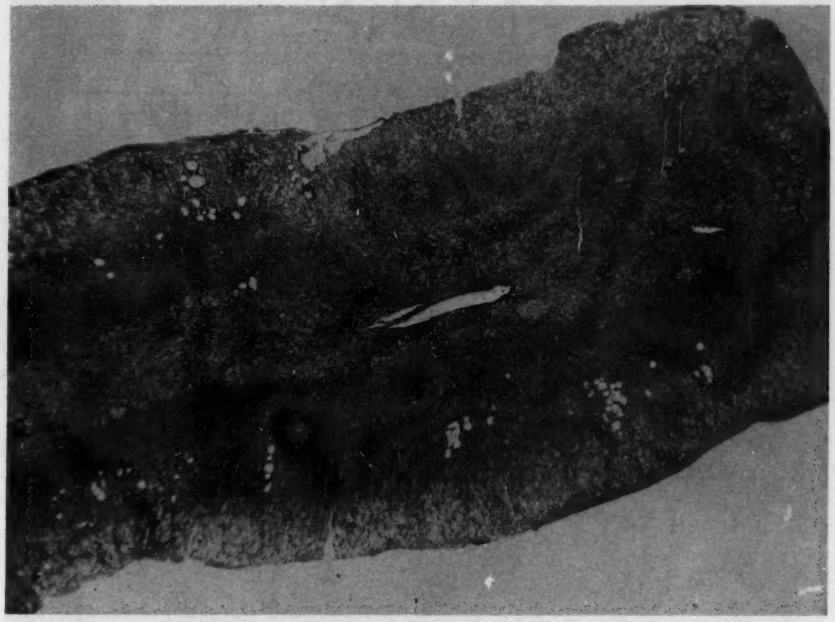
3.6, and globulin 2.1 Gm. per cent. Serum cholesterol concentration was 250 mg. per 100 ml., with a 55 per cent esterified fraction. Serum calcium concentration was 11.5 mg. and 9.4 mg. per 100 ml., and phosphorus was 1.9 mg. per 100 ml. on two determinations. The alkaline phosphatase level was 1.9 Bodansky units; and carbon dioxide combining power, 25 mEq./L. The response to intravenous glucose indicated an impaired tolerance. The blood sugar values after the administration of 35 Gm. of glucose were: 126, 332, 254, 188 and 103 mg. per 100 ml. at 0, 15, 45, 75 and 150 minutes, respectively. The urine was normal and standard urea clearance was 39 ml. per minute. Urinary 17-ketosteroid excretion was 6 mg. per twenty-four hours. Marked calcification along both femoral arteries as well as in the mesenteric nodes, and a calcified mass in the left upper quadrant of the abdomen were reported in the x-ray studies. The spine was markedly osteoporotic and the eleventh dorsal vertebra was narrowed (Fig. 5). There was osteo-

FIG. 5. Roentgenograms of the skull and spine in Case II, showing marked osteoporosis, vertebral compression, and vascular calcification.

porosis of moderate extent in the bones of the lower legs. Intravenous pyelograms were normal. Muscle biopsy was interpreted to show diffuse hyperplastic arteriolosclerosis and increased fat between the muscle bundles. In the biopsy specimen of the skin there was marked atrophy of the dermis with loss of elastic fibers.

Although no definite diagnosis was made, adrenal hypercorticism was considered a possibility. Therapy with methyltestosterone and protein supplements was attempted, but the edema increased markedly. In January 1951 she fell and incurred another laceration of the right knee which ultimately required skin grafting to effect healing. In March 1952 she experienced sudden severe pain in the chest and the electrocardiographic changes were consistent with an acute posterior myocardial infarction. Except for periodic depression, she made an uneventful recovery and returned to work in July 1952. Chest pain recurred periodically and chronic ankle edema persisted despite weekly injections of mercurial diuretics and the administration of digitalis. Her despondency increased to the point that she considered suicide. Largely for this reason, she was hospitalized once more.

The physical findings were striking (Fig. 4). The face was ruddy, without the characteristic "moon" shape. The skin was paper thin, with little subcutaneous fat, and a carotenoid discoloration of the palms was noted. The trunk and extremities were markedly wasted and the lower legs were edematous, with induration and pigmentation prominent in the overlying skin. There was no dorsal hump. Axillary and pubic hair was fine and sparse. The pulse was 84 and the blood pressure ranged between 160/90 and 170/110. There were tiny hemorrhages and scattered old exudates in the fundus of the left eye. There was no palpable thyroid tissue under the thyroidectomy scar. The heart was enlarged; on auscultation of the chest the findings normal. Results of abdominal



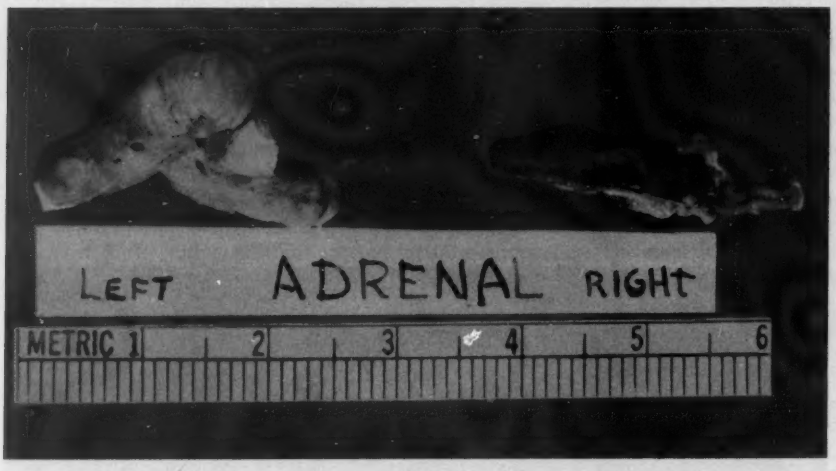
A

FIG. 6. Case II. (**A**) Left adrenal gland, 1937, at the time the clinical diagnosis of Cushing's syndrome was first considered. The gland was grossly and microscopically normal.

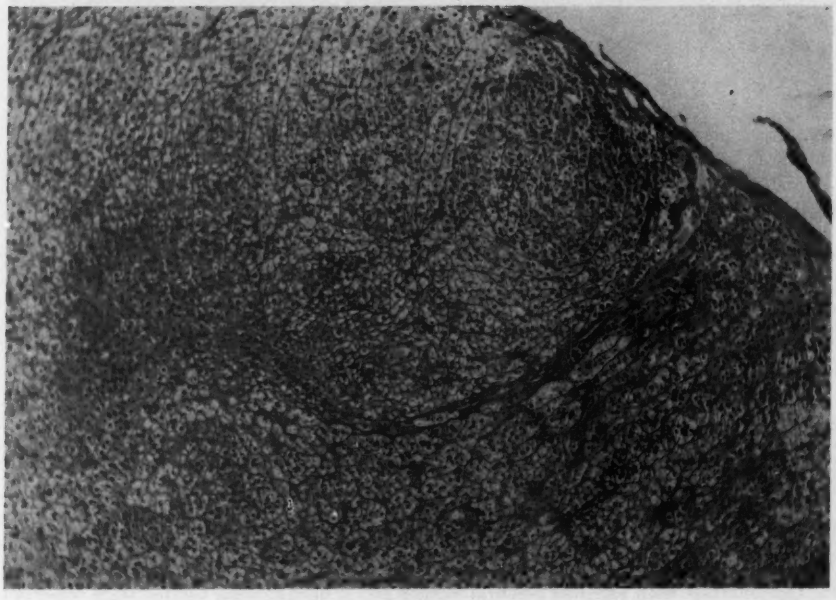
and pelvic examinations were normal, and a neurologic survey revealed only marked muscular weakness.

Osteoporosis with calcified cerebral vessels and a normal sella turcica were observed in the skull roentgenogram. Advanced osteoporosis was also observed in the femora and humeri, which had previously been normal.

The results of the blood count and urinalysis were again normal. The level of serum nonprotein nitrogen was 34 mg. per 100 ml.; calcium 10.8 mg. per 100 ml.; phosphorus 3.0 mg. per 100 ml.; sodium 137 mEq./L; potassium, 3.9 mEq./L; chloride, 98 mEq./L and carbon dioxide combining power, 28.2 mEq./L. Urinary calcium excretion for three consecutive days, while the patient was taking a diet containing 150 mg. of calcium, was



B



C

FIG. 6. Case II. (**B**) The adrenal glands in 1954. The right gland was fragmented, weighed 6 Gm., and appeared normal. The left gland weighed 11 Gm.; the cortex was thickened and multinodular. (**C**) The left adrenal gland, 1954, showing a loss of normal architecture. There are areas of generalized cellular hyperplasia in which the cells are two to three times normal size, with cytoplasm rich in lipid. Adjacent areas show cellular atrophy and fatty infiltration. The right adrenal was normal, its histologic structure resembling the early pattern in the left gland. (H and E ×105)

35 mg., 39 mg. and 52 mg. per twenty-four hours, respectively. The patient's response to intravenous glucose again was impaired, with blood sugar levels of 113, 356, 267, 194 and 126 mg. per 100 ml. at 0, 15, 45, 75 and 150 minutes, respectively. Spontaneous creatine excretion was 566 mg., 448 mg. and 547 mg. per twenty-four hours while the patient was receiving a low creatine-creatinine diet. The titer of urinary gonadotropins was negative on two determinations. However, vaginal smears were interpreted to show normal estrogenic effect, with mature, partially cornified superficial cells.

Observations of urinary steroid excretion were made over several days. Collection periods extended either from 7 A.M. to 7 A.M., or from 7 A.M. to 7 P.M. and 7 P.M. to 7 A.M., the latter schedule allowing observations on diurnal variations in adrenocortical function. After the control period, the patient was given 5 clinical units of corticotropin-gel twice daily for two days. 17-Hydroxycorticoids and 17-ketosteroids were determined as in Case I. The average 24-hour excretion of 17-hydroxycorticoids was 9.9 mg. and of 17-ketosteroids, 12.9 mg. There was little diurnal variation of 17-hydroxycorticoids in urine. A marked increase of hydroxycorticoid excretion followed the low-dose corticotropin stimulation (Table 1).

These data were interpreted as suggesting adrenocortical hyperfunction, and operative intervention was advised. The patient, completely resigned to her illness, accepted the surgical exploration with no hope for improvement. At operation, the right adrenal appeared normal; the left, however, was adenomatous and about twice normal size (Fig. 6 B and C). Bilateral total adrenalectomy was chosen and thirty years after the onset of the original symptoms of the disease the hypercortical state was terminated. No hormonal therapy was given before surgery; in the immediate postoperative period she received 100 mg. of cortisone every twelve hours. Convalescence was complicated by an episode of coronary artery insufficiency, but no specific treatment was required.

On a daily maintenance regimen of 30 mg. of desiccated thyroid, 20 mg. of hydrocortisone and a full diet without added salt, she has steadily improved. The blood pressure is now normal. The edema has disappeared, the strength is returning and the menses continue to be regular. The patient has adopted a more hopeful outlook and has returned to work.

DISCUSSION

The clinical syndromes associated with hyperfunction of the adrenal cortex have been greatly clarified since it has become possible to induce the hypercortical state with corticotropin (ACTH) or to mimic it with adrenal steroid administration. The therapeutic use of these hormones usually requires amounts in excess of that produced endogenously in the normal state. As a result, the syndromes of adrenal hypercorticism are reproduced frequently in clinical medicine. Patients receiving adrenal hormones have been found to differ greatly in their response, some being relatively resistant to the effects, whereas others readily show features ascribed to Cushing's syndrome. This variable response to increased amounts of adrenocortical hormones is dependent to some degree upon the ability of the various organ systems to compensate for the induced physiologic derangements. These in turn are conditioned by such factors as growth rate, nutritional state, disease or other stress conditions. Accordingly, it is more likely that diabetes mellitus will develop in the prediabetic patient (7) or that sustained hypertension will develop in the patient with labile

hypertension, than in persons not so disposed. The administration of corticotropin, cortisone or hydrocortisone has been reported to cause peptic ulcer, psychoses, disseminated tuberculosis, congestive heart failure, epilepsy, hypothyroidism, and many other conditions apart from the commonly known features of Cushing's syndrome. The occurrence of such "side reactions" indicates a latent weakness, which therapy brings to light. This is exemplified by the osteoporosis and vertebral compression fractures which have been observed in patients receiving cortisone therapy for rheumatoid arthritis (8).

Bone atrophy is a commonly observed feature of Cushing's syndrome. Carter and associates (9) found osteoporosis in 10 of 17 cases, and Plotz, Knowlton and Ragan (10) reported this finding in 24 of their 29 patients. However, advanced bone changes and extra-osseous calcification due to adrenocortical hyperfunction are unusual in patients who do not also show obesity, "buffalo hump," "moon" face, diabetes, hirsutism, amenorrhea, or the expected changes in the blood cells and serum electrolytes. In the 2 patients of this report the correct diagnosis could not be established on clinical grounds alone. Indeed, both were suspected of having hyperparathyroidism. The first patient had undergone a fruitless exploratory operation for parathyroid tumor, although the serum calcium and phosphorus levels and the degree of calciuria were not compatible with this diagnosis. The second patient was found on several occasions to have a low concentration of serum phosphorus with a slight elevation of serum calcium. These were not consistently present, however, and urinary calcium excretion was very low.

It is generally accepted that the bone changes induced by excessive adrenal steroids of the 11,17-oxygenated types do not reflect a primary disturbance of mineral metabolism, but are secondary to the protein depletion effects of the hormones. On the other hand, Bartter *et al.* (11) showed that the administration of corticotropin to hypopituitary persons caused an early and significant increase in urinary calcium. Robinson (12) recently reported a patient with Cushing's syndrome and osteoporosis who had hyperplastic parathyroid glands. Removal of these glands resulted in marked hypocalcemia which was followed by recalcification of the bones and a reversal of all the abnormal features of the hypercortical state. The patient previously had received pituitary irradiation which may have affected the outcome. It is possible that the parathyroid glands play a role in the development of osteoporosis in Cushing's syndrome, but it seems likely that this is more a permissive than a causative action. That the demineralization in our cases resulted secondarily from a depleted osteoid, and not from a primary calcium derangement, was suggested by the generalized protein loss apparent in the marked muscle wasting and severely

atrophic skin. Further evidence for this continuing protein depletion was the excessive creatinuria and reduced creatinine index.

The osteoporosis of the postmenopausal years has been attributed to changes in glandular function, chiefly the diminished secretion of gonadal estrogen and androgen. In addition, an alteration in adrenocortical function, characterized by diminished 17-ketosteroid excretion in the presence of normal (young adult) levels of 17-hydroxycorticoids, has been demonstrated in our unpublished studies of a large series of patients with senile osteoporosis. This cortical hormone imbalance is similar to that of Cushing's syndrome and is believed to be of primary significance in the pathogenesis of senile osteoporosis. Amenorrhea is a frequent feature of Cushing's syndrome and a possible deficiency of estrogen or androgen must be considered in any attempt to understand the pathogenesis of such striking bone atrophy. Ovarian hypofunction was not the cause of the bone changes in the female patient of this report, however, since normal menses had been present for over ten years before the diagnosis was finally made, and adequate estrogenic secretion was indicated by the results of the vaginal smear studies. The evidence of normal ovarian function seems inconsistent with the negative urinary gonadotropin assay. Such a negative result has also been obtained in another female patient in whom Cushing's disease appeared during the menopause—when high gonadotropin titers normally are present. In both instances, the gonadotropin titer became normal after adrenalectomy had resulted in a clinical remission. It is our interpretation that the initially low gonadotropin titer may reflect the abnormal protein metabolism of adrenal hypercorticism.

The biologic significance of the adrenocortical hormones which are excreted as 17-ketosteroids has not been fully established. The significance of the 17-hydroxycorticoids, on the other hand, has been well delineated. Albright (13) originally suggested that the "N" hormones, anabolic for protein, were opposed in function by "S" hormones, the 17-hydroxycorticoids. That disorders of this normal steroid balance can occur in patients without adrenocortical tumor is demonstrated by the diminished 17-ketosteroid excretion observed in senility. The adrenogenital syndrome is also characterized by alterations in normal hormone synthesis, usually associated with cortical hyperplasia. In this disorder the urinary 17-ketosteroid excretion is markedly elevated, and the 17-hydroxycorticoid excretion is depressed. The opposite defect, characterized by excessive 17-hydroxycorticoid secretion and reduced formation of the "N" hormones, might reasonably be expected to occur in patients with adrenal hyperplasia. We believe the latter does occur, resulting in Cushing's syndrome, and that the primary defect is of cortical and not of pituitary or hypothalamic origin. Excessive corticotropin, whether of exogenous or endogenous origin,

presumably would induce cortical hyperfunction with maintenance of the normal steroid pattern. Primary adrenocortical hyperfunction, even in the absence of tumor, might be associated with an abnormal pattern of hormone elaboration involving an increase in 17-hydroxycorticoid secretion at the expense of anabolic hormone production. This would explain the extreme bone and muscle atrophy which occurred without hirsutism or sustained amenorrhea in the female patient. Whether or not adrenal androgen secretion was abnormal cannot be stated. Urinary 17-ketosteroid levels do not reliably indicate the amount of adrenal androgen, since a variable fraction is derived from the 17,21-dihydroxycorticoids. However, in both patients the 17-ketosteroid levels were normal in the control periods.

Conclusive evidence that the striking catabolic effects were due to adrenal hyperfunction rested on the demonstration of elevated urinary 17-hydroxycorticoids. Previous observers have reported normal corticoid values in some patients with Cushing's syndrome. These findings may be explained in part by the failure of previous analytical methods to measure total 17-hydroxycorticoids. Our present method (2), a modified Reddy procedure which measures total urinary 17-hydroxycorticoids (1), has proved to be sensitive and reliable. Nonetheless, the determination of the average daily excretion has not clearly distinguished all patients with adrenal hyperfunction of the Cushing's type from normal subjects. The discrepancy has been resolved in part by relating the urinary steroid excretion to both a unit of time and a unit of body mass. This "steroid index," the 24-hour steroid excretion per 1,000 mg. of creatinine, takes advantage of the fact that the patient with Cushing's syndrome loses muscle mass, which lowers the 24-hour creatinine excretion. This diminished creatinine favors higher steroid indices in the Cushing's patient at any level of 17-hydroxycorticoid excretion. In our studies, this index has been higher in all patients with Cushing's syndrome than in the normal group. Of the 2 patients presented here, M.M. (Case I) excreted an average of 25.7 mg. of 17-hydroxycorticoid per twenty-four hours. Since this was considerably above the normal range, the steroid index had no further diagnostic significance. For W.Y. (Case II), however, the average steroid excretion of 9.9 mg. per twenty-four hours was not significantly above normal and the index of 12.8 proved to be decisive.

Further evidence that Patient W.Y. had adrenal hypercorticism is demonstrated by the absence of diurnal variation in the excretion of 17-hydroxycorticoids. Sandberg and associates (14) reported that the ratio between the 12-hour day and the 12-hour night 17-hydroxycorticoid excretion is between 2 and 4. We have confirmed this in normal subjects; but in 7 of 8 patients with Cushing's syndrome whom we have studied, this ratio

has been less than 1.5. We believe this reduction of the normal diurnal variation of steroid excretion is an important diagnostic criterion.

Corticotropin was administered to both patients in a further effort to delineate the origin and the steroid excretion characteristics of the adrenal hyperfunction. It was given in amounts which, in normal subjects, had resulted in a measurable but submaximal increase in 17-hydroxycorticoid excretion (15). Both our subjects exceeded the normal response as measured by total 17-hydroxycorticoid excretion and by steroid excretion per unit of stimulus (Table 1). The dose used was 5 or 10 clinical units twice daily for two days. In this range, Liddle and associates (16) demonstrated a linear relationship between the logarithm of the dose and the adrenocortical response. Assuming that this relationship applies in states of adrenal hyperfunction, the measure of cortical responsiveness may be used to estimate the amount of endogenous corticotropin (ACTH) necessary to maintain basal steroid levels.[1] In Patient W.Y., the calculated endogenous corticotropin secretion of 3.1 clinical units per day was at the lower limit of normal, although her rate of steroidogenesis (steroid index) exceeded normal values. Patient M.M. also responded to corticotropin with a marked increase in steroid excretion and the calculated endogenous corticotropin release of 6.5 units per day was within normal limits. Three other patients with adrenocortical hyperplasia and Cushing's syndrome responded in this manner. One patient with Cushing's syndrome caused by a true adenoma of the left adrenal cortex also responded to corticotropin with the remarkable sensitivity shown by 5 patients with cortical hyperplasia. However, in 1 case, a patient who had adrenocortical hyperplasia associated with a bitemporal quadrantic visual-field defect and slight enlargement of the sella turcica, the elevated urinary 17-hydroxycorticoid excretion was only slightly increased by administration of corticotropin. The urinary 17-ketosteroid level of this patient was not altered significantly by large doses of cortisone—a fact which, according to Jailer and associates (17), indicates the presence of an adrenal tumor. No tumor was found, but this patient presumably had a functional pituitary adenoma, and the calculated endogenous corticotropin was elevated to the high level of 18.9 units per day. This evidence of a distinct difference in the rates of corticotropin release between the patient with a presumed pituitary lesion and the others with adrenal hyperplasia supports the concept that the primary endocrine defect in the latter group was in the adrenal cortex. In fact, these patients

[1] Log endogenous ACTH

$$= \text{basal 17-hydroxycorticoid} \div \frac{\text{17-hydroxycorticoid after ACTH}}{\text{log dose ACTH administered}}.$$

seemed to respond to corticotropin stimulation quantitatively in a manner similar to that in the foregoing patient with a functioning cortical adenoma.

The well-documented history of thirty years of continuous adrenocortical hyperfunction in Patient W.Y. represents one of the most unusual examples of Cushing's syndrome which has come to our attention. Of particular interest were the morphologic alterations that took place in the adrenal cortex during the eighteen-year interval between the original exploration with biopsy and the recent adrenalectomy. In what originally was described as a normal left adrenal cortex there developed over the years striking adenomatous changes (Fig. 6 A, B and C). It is noteworthy that the right adrenal gland was not atrophic, as might be expected with an adenomatous left adrenal. This is further evidence that the primary defect was in the adrenal cortex, for if the abnormality originated in the pituitary, similar changes would be expected in both adrenals. If the adenomatous left adrenal had been the site of a functioning neoplasm, the right adrenal cortex would have become atrophic. Thus, it appears that the derangement was bilateral but was more marked in the left cortex. These changes are similar to those which may be found in the thyroid gland after years of hyperplasia and involution. Most cases of Cushing's syndrome are diagnosed and treated, or the patients succumb to their illness before the development of the unusual clinical and pathologic features observed in Patient W.Y. after thirty years of sustained hypercorticism.

With the wide acceptance of the increasingly reliable steroid analyses in the evaluation of adrenal problems, it is likely that other patients will be found to have excessive 17-hydroxycorticoid secretion which does not result in the complete clinical picture of Cushing's syndrome. It must be recognized that adrenocortical hyperfunction can result in diverse clinical manifestations, ranging from the generalized wasting which characterized these patients to the florid obesity of the classic Cushing's patient. The concept of Cushing's syndrome will require broadening to include these variants as they are detected.

SUMMARY

Case studies of 2 patients with adrenocortical hyperplasia are reported. Both patients presented a clinical syndrome of marked atrophy of the bones, muscles and skin without other features commonly observed in Cushing's syndrome. It is postulated that the adrenocortical hyperfunction in these cases was characterized by excessive 17-hydroxycorticoid excretion with either a relative or an absolute decrease in protein-anabolic adrenal steroids. The urinary 17-hydroxycorticoid level was clearly elevated in only one of the patients. The metabolic derangement was established in the

other by the demonstration of a high 17-hydroxycorticoid-creatinine ratio (steroid index). Both patients responded to small doses of corticotropin with marked increases in 17-hydroxycorticoid excretion. This increased sensitivity of the adrenal cortex is interpreted to mean that endogenous corticotropin levels are normal or low in these cases, and that the primary derangement is probably adrenocortical hyperfunction. In patients with pituitary basophilism, on the other hand, the adrenocortical hyperfunction is thought to be secondary to increased elaboration of corticotropin, and the response to administered corticotropin is not greater than normal. Both patients of this report underwent bilateral total adrenalectomy successfully. Adrenocortical hyperplasia was the pathologic finding in both cases.

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STUDIES ON OBESITY

I. THE INSULIN-GLUCOSE TOLERANCE CURVE

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INTRODUCTION

THE etiology and effects of obesity in the human and in the experimental animal have been the subject of recent investigation (1, 2). To explain possible abnormalities in the metabolism of an obese patient, the authors used a working theory based on the observation that such a patient, whatever the etiology of his unhappy condition, is under continuous mechanical stress. Over a period of years such stress, like any other nonspecific stress, could lead to increased production of endogenous adrenocortical hormones, not necessarily measurable by present methods. This increase could induce gradual changes in metabolism—amongst others, impairment of carbohydrate tolerance and relative depletion of ascorbic acid. Therefore, a study of carbohydrate tolerance in the obese was undertaken, as well as of some aspects of intermediary metabolism (namely, of pyruvic and lactic acid), together with the effect of ascorbic acid on such carbohydrate tolerance following a period of saturation.

Newburgh and Conn (3, 4, 5) feel that the duration of obesity can be correlated with the degree of impairment of carbohydrate tolerance, and that the tolerance would revert to normal in uncomplicated cases of obesity. They do not consider such impairment as a pre-diabetic stage. The opposite viewpoint is held by John (6) who concludes from his studies that the hyperglycemia so often seen in the obese is basically not different from the hyperglycemia of diabetes. Ogilvie (7) states that sugar tolerance may be normal in the earlier years of obesity, but gives way to decreased tolerance which ultimately expresses itself as diabetes. This tolerance is not related to the amount of overweight. Godlowski (8) has classified the obese patient according to insulin sensitivity, and believes that the degree of sensitivity and the duration of obesity cannot be correlated.

Since the results of any of the standard oral glucose tolerance tests are often normal in obesity, especially in the early stage, it was decided to use

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Engel and Scott's modification of the insulin tolerance test, namely the insulin-glucose tolerance test(9). According to Somogyi (10) the administration of glucose thirty to sixty minutes after administration of insulin magnifies the homeostatic response to hypoglycemia. It was hoped that this method of estimating carbohydrate tolerance would yield a better separation of the subjects with decreased tolerance from those who were essentially normal in a group of obese patients. The object was to ascertain whether or not the obese patient followed the biphasic pattern reported in Engel and Scott's series, and whether sensitivity to insulin, height and type of curve could be correlated with the degree of obesity.

METHOD

Procedure. The procedure was identical to the one described by Engel and Scott. (9). The subject is fasted overnight and is kept at rest the following morning. Venous blood is withdrawn and 0.1 unit of regular insulin per kilogram of body weight is injected intravenously. At 30 minutes, or when the first significant symptoms of hypoglycemia appear, depending on which occurs first, a sample of blood is withdrawn and 0.8 Gm. of glucose per kilogram of body weight is given by mouth. Additional blood samples are taken at 60, 90, 120 and 180 minutes from the time of the initial sample. For comparative purposes, the blood sugar levels are reported as a percentage of the initial blood sugar concentration, which is taken as 100 per cent.

Blood sugar levels were determined according to the Folin-Wu method adapted to the spectrophotometer after precipitating the proteins with 10 per cent sodium tungstate (11).

Subjects. Interns, nurses, technicians, and patients hospitalized for elective plastic and orthopedic procedures were used as subjects for the control group. Only patients on full hospital diets were included in the series. The diet contained a maximum of 3,438 calories (proteins 105, fat 190, carbohydrate 327), with the following average daily vitamin intake: vitamin A, 8,570 I.U.; ascorbic acid, 157 mg.; niacin, 15 mg.; riboflavin, 1.7 mg.; and thiamin, 1.6 mg. The other controls ate the regular hospital diet at noon and the remaining meals at home, with emphasis on adequate caloric intake including a minimum of 300 Gm. of carbohydrate daily.

The obese group was taken from the metabolic ward of the hospital and the out-patient department. Each patient was on an unrestricted diet at least two days prior to the test, with a minimum carbohydrate intake of 260 to 300 Gm. Obese diabetics were not included. Our criterion for diabetes was a fasting blood sugar level above 120 mg. per 100 ml., with or without glycosuria. We did not consider that a patient with a normal level of fasting blood sugar and some impairment of glucose tolerance should of necessity be labelled diabetic.

RESULTS

The mean curves of the insulin-glucose tolerance test in 20 normal subjects and 32 obese patients are recorded in Tables 1 and 2 and Figure 1. There was no difference between patients on hospital diets and those who ate variable diets at home. In the majority of cases the biphasic pattern

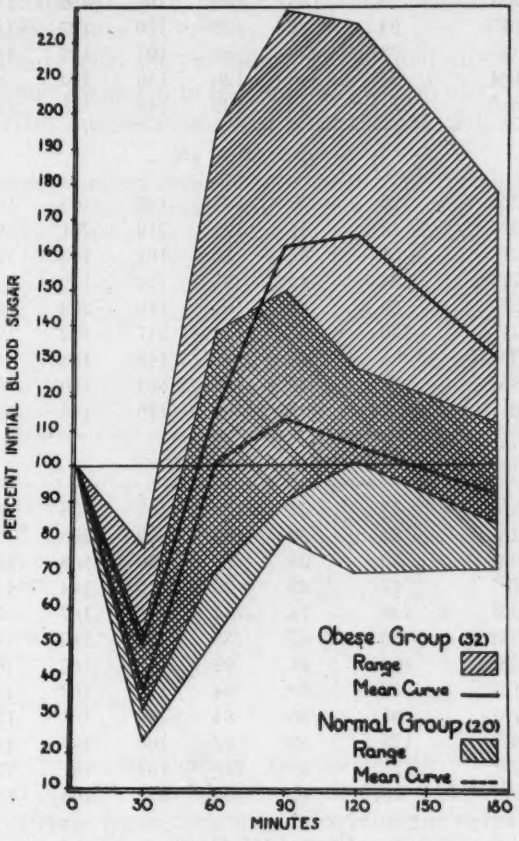

FIG. 1. The insulin-glucose tolerance curve in 32 obese and in 20 normal subjects.

reported by Engel and Scott was apparent (9), although in 5 cases the blood sugar level failed to exceed the initial value at either thirty or sixty minutes after glucose. The range of our results was not as wide, and the peak of the curve not as high as that of Engel's group, probably due to the fact that our controls were not considered normal if their weight deviated more than 15 per cent from that in the standard weight charts of Davenport.

TABLE 1. INSULIN-GLUCOSE TOLERANCE CURVES IN 32 OBESE PATIENTS

Subject			Per Cent Initial Blood Sugar Level					Insulin Reaction
Case No.	Age, sex	Per cent overweight	Minutes					
			30	60	90	120	160	
Group 1: 15–24 yrs.								
1	15M	60	64	163	163	152	113	—
2	19F	94	32	139	150	127	113	—
3	21F	63	58	84	101	130	138	—
4	19M	53	40	120	159	130	84	—
Avge.:	18.5	67.5	49	129	148	135	112	
Group 2: 25–34 yrs.								
5	32M	79	72	161	197	169	143	—
6	33M	43	44	196	219	201	91	—
7	32F	51	47	136	167	163	110	++
8	27F	34	45	95	122	119	95	—
9	32F	59	46	101	170	203	165	++
10	32F	130	53	142	217	182	124	—
11	30M	34	33	79	148	163	113	—
12	28M	38	47	128	164	140	102	—
Avge.:	30.7	58.5	48	130	176	168	118	
Group 3: 35–44 yrs.								
13	36M	43	52	132	193	198	127	—
14	44M	62	50	123	202	225	98	—
15	39M	40	35	70	90	160	130	—
16	37F	47	42	79	119	144	123	—
17	40M	128	74	144	178	187	135	—
18	43M	53	62	133	184	152	112	—
19	36F	103	41	93	140	166	166	—
20	41M	30	63	84	171	162	146	—
21	37M	69	46	84	95	101	177	—
22	40F	128	52	93	166	147	141	—
23	30F	128	58	115	157	151	125	—
Avge.:	39.1	75.5	52	105	154	163	135	
Group 4: 45–55 yrs.								
24	55M	52	36	89	143	175	140	—
25	55M	39	57	99	138	170	110	—
26	47M	129	77	100	162	168	165	—
27	47M	62	75	171	200	209	180	—
28	49M	44	53	100	137	167	125	—
29	45F	74	40	88	161	167	153	++
30	53M	95	46	113	185	211	124	+
31	53M	30	37	112	229	216	137	—
32	51M	42	54	104	125	121	117	—
Avge.:	50.6	63	53	108	164	178	140	
Average value for all obese groups:								
	37.7	*66.7*	*50*	*115*	*162*	*164*	*129*	

Our criterion for obesity was a minimum of 30 per cent overweight, using Davenport's tables corrected for sex and height, and taking 35 years as the optimum age. The range in our study was 30 to 130 per cent overweight. The age limit was 55. Body build was not taken into consideration when computing ideal weight, since only markedly obese patients were studied. Each one was examined for evidence of cardiac failure, liver, renal or chronic respiratory disease.

All obese patients with the exception of 3 showed an exaggerated biphasic pattern when compared to the normal. The first exception was Case No. 8. This patient had become obese after the age of 6 when she had

TABLE 2. SUMMARY OF MEANS, STANDARD DEVIATIONS (S.D.), STANDARD ERRORS (S.E.), AND SIGNIFICANCE OF DIFFERENCES

No. of Cases	Minutes				
	30	60	90	120	180
I. *20 Normal subjects*					
Mean	37	96	114	106	92
S.D.	7.7	24.9	21.1	17.0	10.3
S.E.	1.72	5.57	4.72	3.80	2.30
II. *32 Obese subjects*					
Mean	50	115	162	164	129
S.D.	12.5	30.3	35.6	33.7	24.7
S.E.	2.20	5.36	6.29	5.95	4.38
Diff. between means I and II	13*	19	48*	58*	37*
S.E. of diff. of 2 means	2.80	7.73	7.85	7.06	4.95
III. *8 Normal subjects given excess glucose orally*					
Mean	37	104	135	123	98
S.D.	5.3	21.6	22.3	16.2	10.9
S.E.	1.89	7.62	7.99	5.72	3.85
Diff. between means I and III	0	8	21	17*	6
S.E. of diff. of 2 means	8.09	9.44	9.19	6.86	4.48

* Significant difference at 2.5 times S.E.

contracted poliomyelitis and had to use crutches to walk. The other 2 (Cases No. 3 and No. 21) showed a slowly rising curve with a maximum level at the end of three hours. This may have been due to delayed absorption. The average curve of the obese group was significantly higher than that of the controls, and in half of the patients the final blood glucose concentration did not return to the fasting level. On analyzing the recorded blood sugar values, it was observed that only one curve lay entirely in the range of the normal mean ± 2 standard deviations. In the remaining curves, one or more values were abnormal.

Standard oral glucose tolerance tests were made for the purpose of comparing results with those of the insulin-glucose tolerance test. The curve was considered normal if the peak was not higher than 170 mg. per 100 ml.

and the three-hour blood glucose value returned to, or near the fasting level. Of the 32 obese patients in this study, 9 had abnormal curves, 19 had normal curves, and 4 were not reported.

Only 4 patients showed hypoglycemic symptoms at the end of thirty minutes; 3 of these were females with hirsutism who had been repeatedly investigated for possible Cushing's syndrome. A statistical analysis of the observed insulin reactions is summarized in Table 3.

The obese patients were further divided according to age into four subgroups, namely 15–24, 25–34, 35–44, and 54–55 years. In the younger group, although there was a high average percentage of overweight subjects, there was the least degree of impairment of glucose tolerance. This

TABLE 3. STATISTICAL ANALYSIS OF SIGNIFICANCE OF DIFFERENCE IN NUMBER OF INSULIN REACTIONS OBSERVED IN OBESE AND IN CONTROL GROUP

	No. of insulin reactions		$(0-E)_e$	$\frac{(0-E)_c^2}{E}$
	Observed	Expected		
	No insulin reaction			
Obese	28	17.9	9.6	5.148
Control	0	10.1	−9.6	6.536
	Insulin reactions			
Obese	4	14.1	−9.6	9.124
Control	20	9.9	9.6	9.309
				$\chi^2 = 30.117$

Note: The chances are less than 1:1000 ($\chi^2 = 10.827$) that the difference is due to chance.

might be due to the fact that the average duration of obesity was the shortest. The next age group, namely the one covering 25–34 years, showed a higher peak of blood glucose concentration than the first group, but there was a tendency to return to normal levels at three hours. There was no difference between groups 3 and 4. There was a markedly exaggerated hyperglycemic response after oral glucose and the final blood sugar value tended to be higher than in groups 1 and 2. However, the duration of obesity was 14.6 and 17.4 years in groups 3 and 4, as compared to 6.5 and 9.3 years in groups 1 and 2.

Since one of the main differences between the control and the obese group was the amount of glucose given during the test, it was decided to repeat the test in 8 of the normal subjects, using the same quantity of glucose given to our most obese patients. The mean values with standard devia-

tions and standard errors of the mean are shown in Table 2. No significant difference was observed. In each case the final glucose value returned to the fasting or even below the fasting level.

DISCUSSION

Our results indicate that the insulin-glucose tolerance test is a more sensitive test of carbohydrate tolerance than is the oral glucose tolerance test. All but 1 of our obese patients had one or more blood sugar values outside the normal range during the insulin-glucose tolerance test, whereas in the 28 oral glucose tolerance tests only 9 abnormal values were recorded.

Since the fasting blood sugar levels were translated as 100 per cent and the other values computed accordingly, one might argue that this would introduce a spurious difference in the presence of high or low fasting blood values. In the normal group, initial fasting levels before conversion were in the range of 82 to 113 mg. per 100 ml., with an average of 94 and a standard deviation of ± 6.6, whereas in the obese patient the levels were in the range of 82 to 118 mg., with an average of 98 and a standard deviation of ± 8.0. This difference, though slight, would have the tendency to decrease the final values as reported in the obese and, therefore, to minimize rather than exaggerate the difference between the two groups.

The absence of hypoglycemic symptoms in 28 out of 32 patients, in spite of larger dosages of insulin given on a weight basis, confirms the findings of other investigators. The incidence of relative insensitivity to insulin reported in our experiment and the difference in the blood sugars levels as compared to those in the normal group are statistically significant.

It is difficult to theorize on the exact mechanism for the exaggerated response to ingestion of glucose following hypoglycemia. The maintenance of blood sugar homeostasis is dependent on many factors, notably the anterior pituitary, the adrenal cortex, the adrenal medulla, the glycogen content of the liver, and the rate of absorption from the gastro-intestinal tract. There is no reason to believe that the liver of obese patients is more efficient than normal in storing glycogen, and releasing it as glucose in response to hypoglycemia. The normal homeostatic response to a state of hypoglycemia is perhaps mediated through the pituitary and the adrenal cortex and medulla. The exaggerated response seen in the obese patient (often a high blood sugar level at the end of the three-hour test) has been observed in patients on our metabolic ward treated with 100 mg. or more of corticotropin or cortisone over long periods of time, and has also been reported by Engel and Scott in 5 cases of adrenal and pituitary hyperfunction (9). Such a similarity in response does not, of necessity, imply a common pathogenesis. Exhaustion of the islet cells of the pancreas, with consequent decreased secretion of insulin, especially in patients of ad-

vancing years, might be the explanation of the high blood sugar levels observed after a sudden intake of glucose in spite of normal levels following a regular meal.

SUMMARY

1. The insulin-glucose tolerance curve in the normal subject is compared with that in the obese subject.

2. In 32 obese patients the mean response to the intravenous administration of 0.1 unit of insulin per Kg. followed in 30 minutes by 0.8 Gm. of oral glucose per Kg. consisted of a fall in the blood sugar level to 50 per cent of the initial value followed by a maximum rise to 164 per cent at 120 minutes, and a blood sugar level which was still elevated at the end of 180 minutes. In contrast, the mean response in the 20 normal subjects consisted of a fall to 38 per cent followed by a maximum rise to 114 per cent at 90 minutes and return to a value below fasting level before 180 minutes.

3. The differences in the curves were statistically significant.

Acknowledgments

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STUDIES ON OBESITY

II. BLOOD PYRUVATE AND LACTATE CURVES IN OBESE AND NORMAL SUBJECTS AFTER INGESTION OF GLUCOSE

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RENEWED interest in the metabolism of the obese patient has been evinced by several investigators, and reviews on the subject have been published during the last year. Since pyruvic acid is one of the key breakdown products in the intermediary metabolism of protein, fat and carbohydrate, it was thought that changes in the level of blood pyruvate in response to ingestion of glucose might prove significant. Such a study was carried out on a group of 15 obese patients by Taylor and McHenry, but the results were not statistically different as compared to those in the control group (1). However, since pyruvic acid is so readily reduced to lactic acid, it was considered that such investigations should include determination of blood lactic-acid levels, and that the lactate-pyruvate ratio would give further information as to any deviations in the metabolism of obese subjects as compared to normal ones. Contrary to Taylor and McHenry's view, we theorized that either pyruvic or lactic acid levels would be higher than in the normal, and that there would be a delay in the return to fasting levels. This could be due to the obese person having difficulty in oxidizing pyruvic acid as rapidly as the normal person via the tricarboxylic-acid cycle, with a consequent increase in the concentration of pyruvic and/or lactic acid after ingestion of glucose.

METHODS

In this study 10 subjects of normal weight taken from the orthopedic wards of the hospital acted as controls; they were free of any metabolic disease. The 21 obese patients were from the metabolic ward of the hospital and the out-patient clinic; in them, diseases affecting carbohydrate metabolism, other than obesity, were ruled out. The patients were from 25 to 130 per cent overweight as judged by comparison with the Davenport table of ideal weights. The age of 35 years was used when computing ideal weight for height and sex. The patients received a full hospital diet for at

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TABLE 1. BLOOD GLUCOSE, PYRUVATE AND LACTATE CURVES (MG. PER 100 ML.) IN 21 OBESE AND 10 NORMAL SUBJECTS AFTER INGESTION OF GLUCOSE

	Time after administration of glucose														
	0 minute			30 minutes			60 minutes			120 minutes			180 minutes		
	Glu.	Pyr.	Lac.	Glu.	Pyr.	Lac.	Glu.	Pyr.	Lac.	Glu.	Pyr.	Lac.	Glu.	Pyr.	Lac.
I: *Normal group*															
(10 subjects; mean age 33)															
Mean	94	0.96	11.0	131	1.12	13.4	134	1.25	14.5	121	1.09	13.1	101	0.91	10.9
Standard Deviation:	5.3	0.25	2.4	23	0.26	3.4	14.0	0.30	2.6	7.1	0.23	2.1	14	0.19	1.4
Standard Error:	1.68	0.079	0.758	7.27	0.082	1.08	4.43	0.095	0.82	2.24	0.073	0.664	4.43	0.060	0.443
II: *Obese group*															
(21 subjects; mean age 41.5)															
Mean:	99	1.06	11.7	145	1.25	12.7	169	1.33	14.2	136	1.24	13.9	100	0.96	13.2
Standard Deviation:	7.2	0.29	3.21	22.0	0.33	2.31	34.1	0.30	3.27	28.8	0.28	2.74	23.0	0.20	3.32
Standard Error:	1.57	0.063	0.718	4.79	0.072	0.504	7.44	0.065	0.714	6.28	0.062	0.598	5.02	0.044	0.727
Difference between means of normal and obese groups	5	0.10	0.7	14	0.13	0.7	35	0.08	0.3	15	0.15	0.8	1	0.05	2.3
S.E. of difference of 2 means	2.46	0.10	1.04	8.71	0.11	1.19	8.66	0.12	1.091	6.67	0.10	0.891	6.70	0.74	0.845
Significance at 2.5 times the S.E.	—	—	—	—	—	—	+	—	—	—	—	—	—	—	+

least two days before the test, with a carbohydrate intake of from 260 to 300 Gm. On the morning of the test they were kept fasting and in bed. A blood sample was withdrawn and 1.75 Gm. of glucose per kilogram of ideal body weight was given orally; further samples were taken at 30, 60, 120 and 180 minutes. The patients were warned not to clench their hands during the taking of the samples and the tourniquet was left on for only a minimum period of time, that is, long enough to introduce the needle into the vein. The syringes and specially prepared test tubes for blood pyruvic

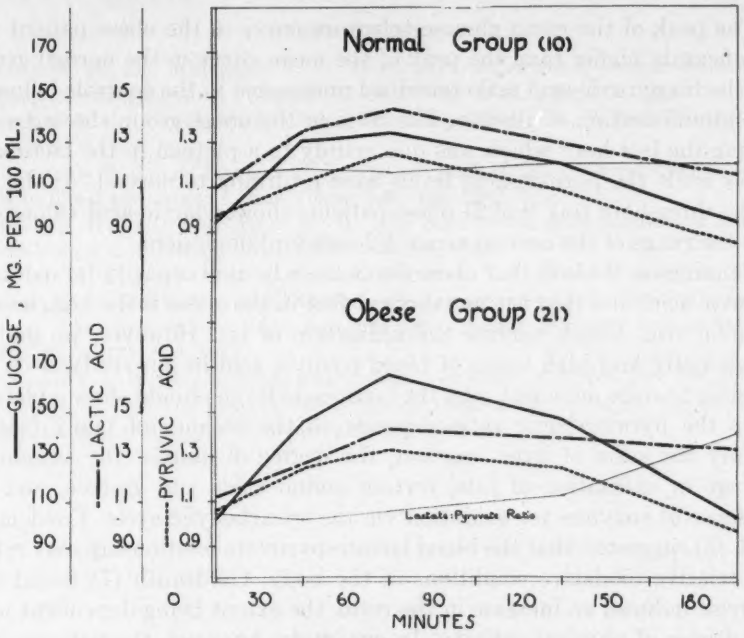

FIG. 1. Mean blood pyruvate and lactate curves in 21 obese and 10 normal subjects, after ingestion of glucose.

acid were refrigerated until the time of use. Blood sugar levels were determined according to the Folin-Wu method adapted to the spectrophotometer after precipitating the proteins with a 10 per cent sodium tungstate solution (2). The estimations of pyruvic acid levels were carried out according to the method of Friedemann and Haugen (3), and those of lactic acid according to the method of Barker and Summerson (4).

RESULTS

The results of the glucose tolerance test, together with pyruvic-acid and lactic-acid determinations and the lactate-pyruvate ratio are shown in

Table 1 and Figure 1. A statistical analysis of the values recorded is included in the table.

Since the pyruvic and lactic values in our 10 control cases were identical to the ones reported by various authors in normal subjects under similar experimental conditions, it was felt that our technique must be comparable to theirs (5). To conserve space, only the means of the control group with standard deviation and error are reported.

DISCUSSION

The peak of the mean glucose tolerance curve in the obese patient was significantly higher than the peak of the mean curve in the normal group. The lactic-pyruvic acid ratio remained unchanged in the controls following the administration of glucose. The ratio in the obese group showed a rise during the last hour which was due mainly to a plateau in the lactic-acid levels while the pyruvic-acid levels were returning to normal. At the end of the three-hour test, 9 of 21 obese patients showed lactic-acid values outside the range of the normal mean ± 2 standard deviations.

Pennington (6) feels that obese tissue has a limited capacity for oxidizing pyruvic acid, and that the metabolic defect in the obese is the high level of pyruvic acid, which inhibits the utilization of fat. However, we did not consistently find high levels of blood pyruvic acid in our study. It is not possible to state definitely why the lactic-acid levels should show a plateau, since the pyruvic-lactic ratio depends on the balance of many factors, mainly the state of liver function, the degree of anoxia, the amount of storage or utilization of fats, certain amino acids and glucose, and the presence of enzymes for oxidation via the tricarboxylic cycle. Friedemann *et al.* (5) suggested that the blood lactate-pyruvate relationship may reflect the relative oxidative conditions of the body. Goldsmith (7) found that exercise induced an increase in the ratio, the extent being dependent upon the degree of physical activity. In our study, however, the patients were confined to bed during the duration of the test. In subjects with clinical evidence of thiamine deficiency, the ratio is decreased. This may also be seen in some patients with riboflavin and niacin deficiency. A repetition of this procedure with concomitant determinations of blood fatty-acid and ketone levels, as well as the oxygen consumption, might give more information as to whether there is a defect in the obese patient's ability to handle the various intermediary products of metabolism.

SUMMARY

1. The blood pyruvic-acid and lactic-acid levels in 21 obese and 10 normal subjects after ingestion of glucose are reported.

2. There was no significant difference between the pyruvate levels in the obese and in the normal subjects.

3. The rise in the lactate-pyruvate ratio in the obese group at the end of three hours was due to a significant increase in the blood lactic-acid level.

Acknowledgments

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DETERMINATION OF TOTAL 17-HYDROXY-CORTICOIDS IN PLASMA*†

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ADRENOCORTICAL steroids are presumably secreted as free, unconjugated compounds. Their urinary metabolites, however, have to a major extent undergone conjugation with glucuronic acid or other substances. Conjugation of the free steroids occurs at least in part in the liver and results in compounds with different physical and chemical characteristics. It is clear that studies of variations in adrenocortical secretion should ideally include measurements of both free and conjugated adrenocortical steroids in both blood and urine.

Methods for the determination of free and conjugated urinary 17-hydroxycorticoids (1, 2, 3), as well as for the determination of free 17-hydroxycorticoids in plasma (3, 4a, 4b) are at present available. The desirability of measuring conjugated corticoids in blood or plasma has been emphasized by the recent demonstration of abnormal rates of disappearance of the free moiety in the presence of hepatic or thyroid dysfunction and possibly of other metabolic disorders (5, 6, 7). Blood levels of free corticosteroids will not reflect the rate of adrenocortical secretion if there are variations in the rate of metabolism and conjugation of these compounds.

Recently Bongiovanni has described a method for measuring conjugated 17-hydroxycorticoids in plasma (7); this method requires considerable time and effort. The present report concerns the determination of total (free and conjugated) 17-hydroxycorticoids in plasma by a method which is relatively simple and can be carried out in one day.

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MATERIALS

1. Sulfuric acid (reagent grade), diluted with an equal volume of distilled water —volume/volume, 50 per cent.
2. *n*-Butyl alcohol (reagent grade).
3. Anhydrous sodium sulfate (reagent grade).
4. Anhydrous sodium carbonate (reagent grade).
5. Ethyl alcohol, (Absolute Pure Ethyl Alcohol, obtained from the U. S.Industrial Chemicals Co.).
6. Phenylhydrazine hydrochloride. Dissolve phenylhydrazine hydrochloride in a minimal amount of absolute alcohol by heating. Allow to cool at room temperature. Then place in refrigerator for at least one hour. Filter through a sintered glass funnel. Repeat this recrystallization. Transfer crystals to a clean container and place in desiccator over calcium chloride.
7. Sulfuric acid, 22 N. To 380 ml. of distilled water in a Pyrex flask, add 620 ml. of concentrated sulfuric acid. Allow to cool. Transfer to a 1-liter volumetric flask and make up to volume with distilled water.
8. Phenylhydrazine-sulfuric acid reagent. Dissolve 65 mg. of phenylhydrazine hydrochloride in 100 ml. of 22 N sulfuric acid. This reagent is stable for at least a week if kept in the refrigerator.
9. Four parts of the phenylhydrazine-sulfuric acid reagent are added to one part of ethyl alcohol.
10. Four parts of the sulfuric acid reagent are added to one part of absolute ethyl alcohol.

Sufficient amounts of reagents 9 and 10 should be freshly prepared each time.

11. Zinc sulfate, 10 per cent. Dissolve 50 Gm. of zinc sulfate (heptahydrate) in distilled water and dilute to 500 ml. in a volumetric flask with distilled water.
12. Sodium hydroxide, 0.5 N. Dissolve 10 Gm. of sodium hydroxide in distilled water and dilute to 500 ml. in a volumetric flask with distilled water.

The two solutions (11 and 12) must be so related that when a 10-ml. sample of $ZnSO_4$ is titrated with NaOH, using phenolphthalein as an indicator, the pink end-point comes when the amount of added alkali is between 10.9 and 11.2 ml. (The 10-ml. sample of $ZnSO_4$ is diluted to about 50 ml. with distilled water before titrating with the alkali.)

PROCEDURE

A. *Estimation of total 17-hydroxycorticoids*

Blood samples are collected with heparin and the plasma separated from the red cells within thirty minutes. To 10 ml. of plasma in a 25-ml. glass-stoppered centrifuge tube are added 5 ml. of 10 per cent zinc sulfate and 5 ml. of 0.5 N sodium hydroxide. After thorough mixing and centrifugation for thirty minutes at 3,000 r.p.m., the supernatant material is decanted into a clean 25-ml. glass-stoppered tube, and the pH is adjusted to 1 (indicator paper) by the dropwise addition of 50 per cent (vol./vol.) sulfuric acid. Then are added 2.5 to 3.0 Gm. of Na_2SO_4, followed by 10 ml. of *n*-butanol, and the extraction is carried out by shaking vigorously for five minutes. After centrifugation for five minutes at 2,500 r.p.m., as much of the butanol as possible is transferred to a clean test tube (by

means of a capillary pipette to which a rubber bulb is attached). Next, 0.25 Gm. of anhydrous sodium carbonate is added to the butanol extract and thoroughly shaken (if cork stoppers are used they should be covered with parafilm). The mixture is allowed to stand for five minutes, and then centrifuged for five minutes at 2,500 r.p.m. After being decanted into a clean test tube, two 4-ml. portions of the butanol extract are transferred into two clean 25-ml. Erlenmeyer flasks (A and B).

The evaporation is carried out by suspending the Erlenmeyer flask in a water bath which is at a temperature between 90° and 95° C. After drying, 0.8 ml. of reagent #9 is added to each A flask, and 0.8 ml. of reagent #10 to each B flask. The contents of the flasks are swirled to dissolve the residue and then the solutions are transferred as quantitatively as possible to test tubes. The tubes, along with similar tubes containing suitable standards and blanks, are placed in a water bath at 60° C. for twenty minutes. The samples are then placed in cold water for three minutes, after which the contents are transferred to microcuvettes and read in the Beckman spectrophotometer at a wave length of 410 millimicrons.

B. *Estimation of free and conjugated 17-hydroxycorticoids*

Both the free and the conjugated moieties may be measured in the same sample by first determining the plasma 17-hydroxycorticoids by either the method of Eik Nes *et al.* (4b) or that of Silber (3), and then carrying out the procedure described in the present paper for total 17-hydroxycorticoids on the chloroform-extracted plasma. In both of the methods, chloroform is used to extract the free 17-hydroxycorticoids.

EVALUATION OF THE METHOD

Precipitation of proteins

Since in previous experiments it had been observed that pretreatment of urine with zinc sulfate and sodium hydroxide did not result in any loss of 17-hydroxycorticoids, the protein precipitation technique of Somogyi (8) was investigated for its applicability to plasma. Recovery experiments were carried out for free and for conjugated 17-hydroxycorticoids. For the recovery of free steroids 5-ml. specimens of plasma were used, to which known amounts of cortisone had been added. The cortisone solutions were prepared by diluting an absolute-alcohol solution containing 1 mg. of cortisone per ml. with distilled water, resulting in aqueous solutions containing the desired concentration of cortisone. The recoveries are shown in Table 1; they ranged from 94 to 110 per cent.

Since pure 17-hydroxycorticoid conjugates are not available, urinary conjugates were used to evaluate the recovery from plasma. A urine specimen was thoroughly extracted with chloroform at pH 1 in order to remove

TABLE 1. RECOVERY OF FREE 17-HYDROXYCORTICOIDS FROM PLASMA

Amt. of cortisone added (μg.)	Amt. of cortisone recovered (μg.)	Per cent recovered
5	4.7	94
5	6.0	120
10	9.6	96
10	10.0	100
20	20.0	100
20	21.0	105

the free steroids, and then extracted with butanol. After evaporating the butanol extract to dryness, the residue was dissolved in ethanol and the amount of Porter-Silber chromogen was determined. Measured amounts of this solution were evaporated and in each instance the dried residue was dissolved in 10 ml. of plasma, in which it was easily soluble. Total steroid determinations were then carried out and recoveries ranged from 92 to 106 per cent, as shown in Table 2.

Extraction procedure

The extraction procedure is similar to that previously used for urine (1) although, because of the small concentration of steroid in plasma, all of the butanol extract has to be used and concentration by evaporation becomes necessary. Evaporation to dryness at elevated temperatures has been usually considered to bring about changes in the reactive groupings

TABLE 2. RECOVERY OF CONJUGATED 17-HYDROXYCORTICOIDS FROM PLASMA

Amt. of conjugate added (μg.)	Amt. of conjugate recovered (μg.)	Per cent recovered
4	3.7	93
4	4.2	105
8	8.0	100
8	8.5	106
12	11.0	92
12	12.2	101
18	18.5	103
18	18.5	103
36	34.8	97
36	35.5	99

of steroids. These changes have been attributed to increased temperature or to the presence of oxygen; alterations of the steroid molecule might also be accounted for by changes in pH during evaporation of mildly acid or alkaline solutions,—changes which can be avoided by the use of weak acid or base. Decomposition of the solvent being evaporated might also account for steroid losses. Chloroform has been the most commonly used solvent for the extraction of free corticosteroids and it is known to have destructive properties if not freshly distilled. Experiments have been carried out to ascertain whether the evaporation procedure results in any appreciable steroid loss. The data shown in Table 3 indicate that evaporation of butanol solutions of free steroids and of butanol extracts of urine at tem-

TABLE 3. EFFECT OF EVAPORATION AT ELEVATED TEMPERATURE (90–95° C.) OF BUTANOL SOLUTIONS OF 17-HYDROXYCORTICOIDS

Butanol solutions of pure compounds (μg./ml.)	Amount present after evaporation (μg./ml.)	Difference	Butanol extract of urine (μg./ml.)	Amount present after evaporation (μg./ml.)	Difference
10.0 Cortisone	9.8	−0.2	2.2	3.0	+0.8
10.0 Cortisone	10.4	+0.4	5.7	6.1	+0.4
10.0 Cortisone	10.1	+0.1	6.4	5.3	−1.1
10.0 Hydrocortisone	10.0	0.0	9.5	9.1	−0.4
10.0 Hydrocortisone	10.0	0.0	13.2	14.2	+1.0
10.0 Hydrocortisone	10.1	+0.1	22.0	20.8	−1.2

peratures between 90 and 100° C. does not result in any significant loss of steroids as measured by the Porter-Silber reaction.

Nature of the conjugated steroids measured

Samples of plasma were obtained from the hospital blood bank and the proteins were removed by precipitation. The free steroids were extracted with ethyl acetate. The residue was then hydrolyzed with β-glucuronidase and re-extracted with ethyl acetate. This solution was washed with 0.1 N sodium hydroxide and water and then evaporated to dryness. The residue was chromatographed on paper along with pure standards, using the toluene-methanol-water system of Bush (9). The chromatogram was developed with blue tetrazolium and was found to contain mainly a compound with an Rf value identical with that of tetrahydrohydrocortisone. Elution of an unstained portion of the small area of the chromatogram showed the Porter-Silber reaction. Further identification was not carried out.

A comparison of the spectral curve obtained with cortisone and that obtained with butanol extracts of plasma in the Porter-Silber reaction is illustrated in Figure 1.

Clinical evaluation

With the procedures available to date for the determination of steroids, complete and repeated identification of the compounds being measured is not practically feasible. When attempting to evaluate the usefulness of a procedure for clinical and physiologic purposes it is therefore desirable and necessary to establish the degree of correlation of the findings with known

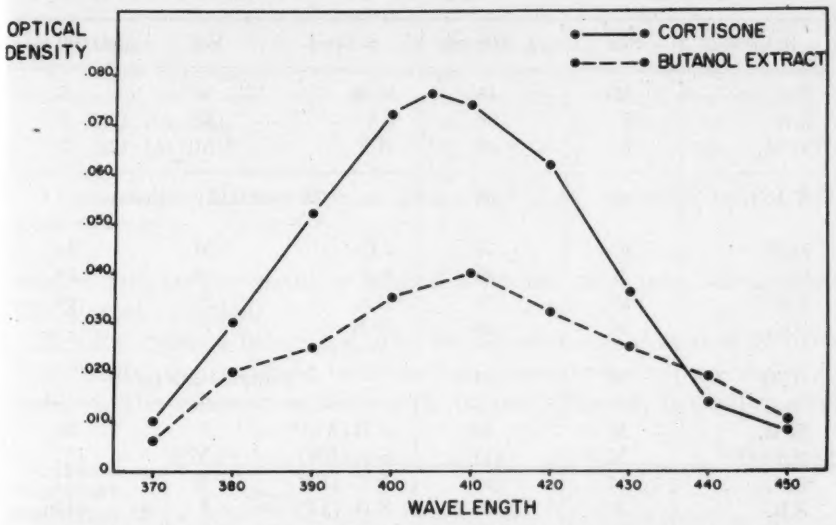

FIG. 1. Comparison of the spectral curve obtained with cortisone and that obtained with butanol extracts of plasma in the Porter-Silber reaction.

states of adrenocortical function. Definitive appraisal then has to await prolonged clinical use.

A limited number of normal subjects were used to establish the tentative range of basal morning levels shown in Table 4. Also shown are the levels obtained in patients with Addison's disease, a patient with hypopituitarism, and patients with Cushing's syndrome. When the results in these patients are compared with the normal range, a clear-cut differentiation is observed.

It has been previously shown that there is a diurnal variation in the level of both free 17-hydroxycorticoids in plasma (10) and urinary 17-hydroxycorticoids (11). The values obtained in 5 normal males on samples

taken at 8:30 A.M. and 5 P.M., as well as the values in a normal individual both on a control day and before and after the intravenous administration of 25 units of corticotropin (ACTH) over an eight-hour period, are shown in Table 5. The importance of the control value selected when evaluating an adrenocortical response is apparent. A more detailed study of the

TABLE 4. MORNING LEVELS OF TOTAL 17-HYDROXYCORTICOIDS IN PLASMA OF RESTING SUBJECTS WITH NORMAL AND ABNORMAL ADRENAL FUNCTION

NORMAL SUBJECTS			ADDISON'S DISEASE		
			Untreated		
Subject	Sex	μg./100 ml.	Subject	Sex	μg./100 ml.
H.P.	M	45	H.W.	M	6
L.B.	F	69	J.S.	M	2
M.M.	F	40	H.F.	M	2
N.A.H.	M	66	On maintenance therapy		
M.W.	F	56	J.C.	M	24
B.S.	M	30	E.C.	M	34
P.B.	M	78	R.S.	F	20
F.G.	M	28	M.S.	F	12
W.M.	M	33	CUSHING'S SYNDROME		
W.R.	M	45	P.B.(AA)*	F	93
R.F.	M	111	S.S. (BH)	M	187
S.G.	M	38	R.C. (BH)	M	171
S.R.	F	93	S.D. (AC)	F	414
L.E.	F	44	HYPOPITUITARISM		
			A.V.	F	0

* AA = Adrenal Adenoma.
BH = Bilateral Hyperplasia.
AC = Adrenal Carcinoma.

diurnal variation in plasma corticoid levels was carried out by taking samples at four-hour intervals over a 24-hour period in a normal subject. The results are shown in Figure 2 and it is apparent that the level of total 17-hydroxycorticoids in the normal individual is definitely related to the time of sampling. Therefore, the evaluation of changes in total 17-hydroxycorticoids in plasma under conditions of stress or during pharmacologic

TABLE 5. DIURNAL VARIATION OF PLASMA 17-HYDROXYCORTICOIDS IN NORMAL SUBJECTS

Subject	17-OH-CS (μg./100 ml.)	
	8:30 A.M.	5:00 P.M.
B.S.	30	19
P.B.	78	19
F.G.	28	18
W.M.	33	15
W.R.	45	18
R.F.	62	19
Range	*28–78*	*15–19*
Mean	*46*	*18*
W.J. (Control)	45	18
W.J. (ACTH)*	47	150

* Corticotropin (ACTH)—25 units administered in an intravenous infusion from 9 A.M. to 5 P.M.

studies with corticotropins or adrenal hormones must take into account this diurnal variation.

Results obtained before and after the intravenous infusion of 25 units of corticotropin over eight hours on two consecutive days are shown in Table 6. The values in patients with Addison's disease, hypopituitarism

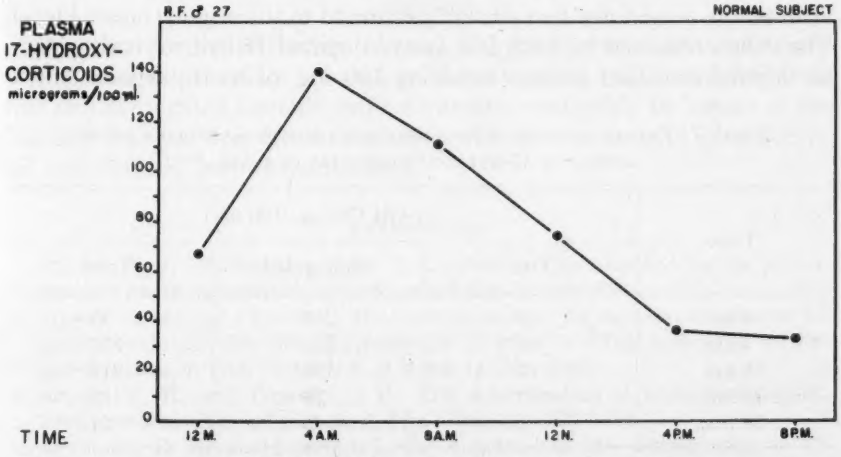

FIG. 2. Diurnal variation in the plasma level of total 17-hydroxycorticoids in a normal subject.

TABLE 6. PLASMA LEVELS OF 17-HYDROXYCORTICOIDS BEFORE AND AFTER INTRAVENOUS INFUSIONS OF 25 UNITS OF CORTICOTROPIN OVER EIGHT HOURS ON TWO CONSECUTIVE DAYS

Subjects	17-OH-CS (μg./100 ml.)			
	First Day		Second Day	
	0 hr.	8 hrs.	0 hr.	8 hrs.
Normal	93	150	53	155
Addison's disease*	20	17	25	30
Hypopituitarism	0	0	19	52
Cushing's syndrome	180	235	264	320

* This patient was being maintained on 25 mg. of cortisone per day prior to, and during the period of observation.

and Cushing's syndrome are compared with those in a normal subject. The Addisonian patient was being maintained on 25 mg. of cortisone per day and no response to corticotropin could be demonstrated. The patient with hypopituitarism showed no response on the first day of corticotropin administration but did show a definite response on the second day. The patient with Cushing's syndrome showed a markedly elevated control level and, in addition, a persistence of the response to corticotropin on the morning following the first infusion of this hormone at a time when the plasma 17-hydroxycorticoids in the normal subject had returned to a value below the original control level. In other normal subjects the control level on the second day had similarly returned to the original control level. The values obtained for both free and conjugated 17-hydroxycorticoids in an adrenalectomized patient receiving 100 mg. of hydrocortisone intra-

TABLE 7. EFFECT OF INTRAVENOUS INFUSION OF HYDROCORTISONE ON THE LEVELS OF 17-HYDROXYCORTICOIDS IN PLASMA*

Time	17-OH-CS (μg./100 ml.)		
	Free	Conjugated	Total
0 hr.	18	57	75
4 hrs.	105	272	377
10 hrs.	62	150	212
12 hrs.	37	78	115
24 hrs.	8	43	51

* Hydrocortisone was given intravenously in a dose of 100 mg. over an 8-hour period beginning at 0 hour.

venously over eight hours are shown in Table 7. It would appear that the levels of free and conjugated steroids varied in essentially parallel fashion.

The method described is, with slight modifications, analogous to that previously described for urine. Chemical identification of the material being measured constitutes a greater problem in plasma than in urine because of the limited amount of material available. However, the spectral curves obtained with unknown extracts when using the relatively specific Porter-Silber reaction, and the correlation demonstrated with known states of adrenocortical function constitute strong evidence for the identity of the material being measured. One must remember, nevertheless, that in special situations interfering chromogens may be present, due to either administration of, or endogenous production of, abnormal compounds which are not excluded by the precipitation, extraction or evaporation procedures.

Selection of a reference standard in any method in which a mixture of compounds is being measured is arbitrary, especially when the extinction coefficients are different for each compound. The best that can be done under these circumstances is to select as a reference standard that compound which normally constitutes the greatest fraction of the mixture measured. Although it has been demonstrated repeatedly that hydrocortisone is the major component of the free fraction and tetrahydrohydrocortisone is the major component of the conjugated fraction, we arbitrarily selected cortisone as our reference standard, both because of its availability and our previous experience with it.

SUMMARY

A method for the estimation of total 17-hydroxycorticoids in plasma is described. The procedure is based on the extraction with butanol of both free and conjugated steroids and colorimetric evaluation by means of the Porter-Silber reaction. Good correlation with known states of adrenocortical function has been demonstrated.

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TOXICITY OF THE ANTITHYROID DRUG CARBIMAZOLE

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IN THE twelve years which have elapsed since the introduction of thiourea in the treatment of thyrotoxicosis, other antithyroid drugs have replaced it in turn, each introduced in the hope that it would prove equally efficacious but less toxic. One of the more recent is 2-carbethoxy-thio-1-methyl-glyoxaline (syn., carbimazole B.P.; Neo-mercazole) first introduced by Lawson, Rimington and Searle and later reported by Macgregor and Miller (1–2). The effects in patients suffering from thyrotoxicosis were first described by Lawson and Barry (3) who, in their first 14 patients, did not observe any untoward reactions. Clinical reports have since been published by Poate, Doniach, Bartels, Richardson *et al.*, Harrison, and Fraser *et al.* (4–9).

The object of the present investigation, begun before this evidence was available, was to satisfy ourselves that in carbimazole we possessed a drug superior to methylthiouracil, which had previously been used at New End Hospital.

METHYLTHIOURACIL

The toxicity of methylthiouracil has been repeatedly examined. Van Winkle *et al.* reported untoward effects in 13 per cent of 5,745 cases; Barfred in 7 per cent of 604 cases; Dunlop and Rolland in 11 per cent of 200 cases; Bartels and Ingham in 15 per cent of 100 cases; and Greene in 6.5 per cent of 200 cases (10–14). Since the last of the foregoing data were collected, a further 261 patients have been treated with methylthiouracil (200 mg. twice daily) at New End Hospital, as the sole treatment in 31, as a preliminary to radioiodine in 9, and in preparation for surgery in 221. Untoward effects were observed in 4.6 per cent. These included 6 cases of agranulocytosis (2.6 per cent). Only 1 case of leukopenia was observed. It should, however, be emphasized that routine blood counts are not performed in our clinic, and that therefore some cases of transient leukopenia may have been unobserved. Four patients had skin reactions; 1 suffered

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severe nausea; and 1 had a sore throat which recurred at each exhibition of methylthiouracil. All reactions occurred in the first forty days of treatment. There was 1 death—from agranulocytosis.

In combining these figures (Table 1) some selection has been exercised. Certain reactions have been reported so seldom that their association with the administration of any particular drug must be called in question: such are mentioned here only if they were serious enough to cause abandonment of treatment. When, too, a rare reaction has been mentioned by only one author (*e.g.*, 4 cases of jaundice in Van Winkle's collection of over

TABLE 1. REACTIONS TO METHYLTHIOURACIL

Authors	No. of cases	Miscellaneous rashes	Purpura	Agranulocytosis
Van Winkle *et al.*	5,745	189	6	142
Barfred	61	13	0	1
Dunlop and Rolland	200	3	0	2
Greene	200	7	0	2
Bartels & Ingham	100	5	0	1
Greene & Morgan	261	4	0	6
Total No. of cases	6,567	221	6	154
		3.4%	*0.1%*	*2.3%*
		Total reactions—5.8%		

5,000), it has been omitted in order not to confuse an otherwise clear comparison between different series.

CARBIMAZOLE

The next 181 patients needing antithyroid drugs were treated with carbimazole, as the sole therapy in 32 cases, as a preliminary to radioiodine in 17 and in preparation for thyroidectomy in 132. The dose used was 10 mg. three times daily. Toxic effects occurred in 8 cases (4 per cent) (Table 1). Of these 8 patients, 4 had harmless rashes which did not necessitate a change of treatment; 2 had purpura (1 with psychotic depression and 1 with sore throat and pyrexia); 1 had pretibial myxedema (also with depression); and 1 had a pure red cell anemia. There were no deaths. The last 3 cases are worthy of detailed report.

Case 1

A 48-year-old man complained of loss of weight, vomiting and muscular weakness for the preceding nine months. Examination revealed a regular pulse of 100, tremor and mild myopathy. Lid lag and retraction were present on the left but there was no exophthalmos. He was considered to be suffering from a moderately toxic, diffuse goiter.

Reporting two weeks after the beginning of treatment, he said that four days after commencing carbimazole his lips, ankles and feet had become swollen, and there had been an irritation over the lower third of his legs. He was extremely depressed and wept profusely. His pulse was 80. There was tense non-pitting edema of the lower third of his legs. The drug was changed to methylthiouracil. His mental condition improved, but two weeks later he had typical pretibial myxedema. When seen four months after thyroidectomy he felt well and had regained his strength and weight. Pretibial myxedema was still present.

Case 2

A 48-year-old female complained of attacks of palpitation which had previously been diagnosed as due to auricular fibrillation. This diagnosis was confirmed clinically and by electrocardiogram. She was found to be suffering from a moderately toxic diffuse thyroid enlargment. Two weeks after treatment with carbimazole began she had gained $6\frac{1}{2}$ pounds and said she was feeling much better. When seen one week later she confessed

TABLE 2. REACTIONS TO CARBIMAZOLE

Authors	No. of cases	Rashes	Pretibial myxedema	Purpura	Agranulocytosis	Pancytopenia	Pure red cell anemia
Lawson & Barry	14	0	0	0	0	0	0
Poate	9	0	0	0	0	0	0
Richardson	1	0	0	0	0	1	0
Harrison	1	0	0	0	1	0	0
Bartels	52	3	0	0	1	0	0
Fraser	57	0	0	0	1	0	0
Doniach	120	0	0	0	0	0	0
Greene & Morgan	181	4	1	2	0	0	1
Total No. of cases	435	7	1	2	3	1	1
		1.6%	*0.2%*	*0.5%*	*0.7%*	*0.2%*	*0.2%*
				Total reactions—3.4%			

that she had been feeling very depressed a week before her previous visit. The depression had continued. She felt "like a sack of potatoes; venomous; could murder my best friend." She was weeping throughout the interview. There were numerous purpuric spots across the bridge of her nose and below her eyes. Hess's test yielded positive results. The total white cell count was 10,300. The platelet count was normal. She was given methylpentynol (500 mg. twice daily) and promethazine hydrochloride (25 mg. twice daily) and recovered in a few days. After a two-week course of Lugol's iodine (5 minims three times daily) subtotal thyroidectomy was carried out by Mr. Piercy. Her recovery was uneventful.

Case 3

A woman aged 60 complained in March 1953 of extreme dyspnea and was found by her family doctor (Dr. Barnet Solomons) to have auricular fibrillation and congestive heart failure. She had a nodular goiter of moderate size, known to have been present for some years. She was treated with mersalyl and digitalis and made a complete recovery from her cardiac disorder. In September 1953 cardiac failure again developed, with severe edema; consequently her weight rose rapidly from 161 to 175 pounds. Further treatment induced rapid alleviation, the weight dropping to 153 pounds—8 pounds lower than her weight before the onset of failure.

When first seen by R.G. on October 5, 1953, there were no signs of cardiac failure. Auricular fibrillation was present, but the rate was well controlled with digitalis. Her skin was slightly warm and damp and her hands tremulous. The mucous membranes were of normal color. The additional drop in weight was observed. It was considered that her goiter had become toxic and that thyroidectomy was advisable.

Treatment with carbimazole was begun with a dosage of 10 mg. three times daily, but after further consultation next day it was decided that preparation with iodine would be sufficient and the drug was stopped after only 30 mg. had been administered. During the next twelve days she became somewhat pale and a blood count on October 19 showed that she had a hemoglobin of 7.2 Gm. per cent, with only 2.1 million red cells per cu. mm. The white cell count (4,400 per cu. mm.) was in normal proportions, the platelets numbered 170,000. Reticulocytes constituted 5 per cent of the RBC. She was admitted immediately to the Royal Northern Hospital where her hemoglobin was found to have dropped to 35 per cent. Marrow puncture showed complete aplasia of the red cell precursors with normal white cell formation (Dr. Martin Hynes). The direct Coombs test yielded negative findings. Prothrombin content was normal. Results of liver function tests were all normal.

The cause of her anemia being somewhat obscure and her condition desperate, it was thought advisable to treat her from all angles. She was given intravenous iron, folic acid and cyanocobalamin as well as blood transfusions, without any effect upon the rate of fall of her red cells. Courses of soluble corticotropin intravenously and of corticotropin gel intramuscularly were ineffective in slowing the rate, but coincidently with (though not necessarily because of) their administration, signs of thyrotoxicosis, apart from auricular fibrillation, disappeared. On January 26 splenectomy was performed, with some beneficial effect. Before operation, it was necessary to transfuse an average of a pint of blood every 2.5 days; after operation she needed a pint every 9.3 days. Before the operation the rate of fall of hemoglobin suggested active and abnormal blood destruction; postoperatively, the rate was typical of pure aplasia.

As Chalmers and Boheimer (15) had reported a case of pure red cell anemia which had failed to respond to corticortropin before splenectomy but responded after the operation, a further course was given in March 1954, but no response occurred. During the summer of 1954, with the aid of transfusion, her general health remained fairly good and the question of reduction of thyroid function was again discussed. Radioiodine was barred because of its possible effect on her bone marrow. In September a thyroidectomy was successfully performed by Mr. Alan Hunt. Microscopy of the gland showed, in general, a nontoxic nodular goiter with no epithelial hyperplasia. After operation, though fibrillation persisted, the heart rate became slower and the rhythm more nearly regular. The anemia was unaffected but she continues to live in moderately good health with the aid of blood transfusions and a small dose of thyroid.

DISCUSSION AND SUMMARY

It would appear that the only effects of antithyroid drugs which need arouse grave concern are those upon the bone marrow. These are agranulocytosis, pancytopenia and pure red cell anemia. Only 1 case of each of the last two disorders has been recorded, both in patients treated with carbimazole, but it is impossible to prove that they were due to the drug administered. The coincidence in time makes it probable, though the minute dose of carbimazole administered to our patient with pure red cell anemia

throws doubt on the cause-effect relationship. Even assuming such a relationship, carbimazole emerges from this enquiry as a safer drug than methylthiouracil, causing serious side effects in 1.1 per cent of patients compared with 2.3 per cent of patients treated with methylthiouracil.

If lesser reactions are brought into the picture, carbimazole has still the advantage, with 3.4 per cent total reactions compared to 5.8 per cent following methylthiouracil. Another possible advantage has been suggested by Lawson and Barry and by Poate. Their suggestion that the thyroid glands of patients prepared for operation by carbimazole are less congested than those of patients prepared by methylthiouracil, and therefore easier to remove, is supported by the opinions of Mr. J. E. Piercy and Mr. M. J. Lange, the surgeons who have operated on the patients in this series. Against this must be set a minor disadvantage, again supported only by opinion. It has appeared to the physicians and surgeons alike at New End Hospital that the beneficial effects of carbimazole are less lasting than those of methylthiouracil. On several occasions, domestic or administrative difficulties have led to operations being performed a week or two later than the planned date. In consequence, the administration of carbimazole was stopped and the substitution of iodine begun, some four weeks before admission for operation instead of the usual two weeks. When the preparation had been carried out with methylthiouracil the delay appeared to make no difference. Since the use of carbimazole, several patients have been observed in whom thyrotoxicosis has reappeared before operation; convalescence, in consequence, has been less smooth. In one or two instances the relapse has been of such severity that operation has been postponed and a further course of carbimazole instituted. It has recently been decided that carbimazole should be continued in every case until seven to ten days before operation, iodine treatment in consequence slightly overlapping the treatment with carbimazole.

Despite this slight disadvantage, we are of the opinion that carbimazole is superior to methylthiouracil, both as the sole treatment in selected cases and as a preparation for thyroidectomy.

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PITUITARY TUMOR WITH GYNECOMASTIA AND LACTATION.

CASE REPORT

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IT HAS been known for some time that secretion may be present in gynecomastia. However, there are few references in the literature to actual observation of a fluid, either flowing spontaneously or by expression, from the male breast. In some cases the secretion is watery clear or yellow; in others it is a mucinous or a fatty liquid material. In a few cases a "milky fluid" has been found. One such case has been reported in which the patient had a pituitary tumor and acromegaly (1); gynecomastia was not present but the nipples projected and a milky fluid could be expressed by moderate pressure. The second case of gynecomastia with lactation was associated with adrenocortical tumor (2). In the third case no pathologic condition could be demonstrated in the pituitary, the adrenals, or the testes, but the striking feature was bilateral gynecomastia with persistent lactation (3).

The following report of a case of gynecomastia with persistent lactation in a young man is presented because of the rarity of the condition. Our case differs from the cases mentioned, in that the patient had a pituitary tumor but manifested no changes of acromegaly, and in that there were no other clinical or laboratory findings indicative of other endocrine malfunction.

CASE REPORT

J.B.C., (Fig. 1) a white man, 26 years of age, was admitted because of complaints of recurrent dull occipital headaches and enlargement of both breasts which was first noted eight years before. The patient had become aware that at times a white milky fluid could be expressed from the nipples of the enlarged breasts. He stated that during the preceding eighteen months he had had an excessively large appetite, had gained 45 pounds (from 190 to 235 pounds), and had slept poorly. There was no impairment of vision, and no history of polydipsia or polyuria. He thought his beard was scanty, and

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he believed that his penis always had been smaller than normal. Erections were infrequent and he averred that he had never had sexual contact. His father was thought to have diabetes mellitus and one sister was said to have "gland trouble" (?). The medical history was non-contributory.

Initial physical examination: The patient's height was 5 feet 9 inches; weight, 235 pounds; pulse rate, 80/minute; and blood pressure 140/100 mm. Hg. The obesity was generally distributed, but there was some excessive deposition on the upper chest and

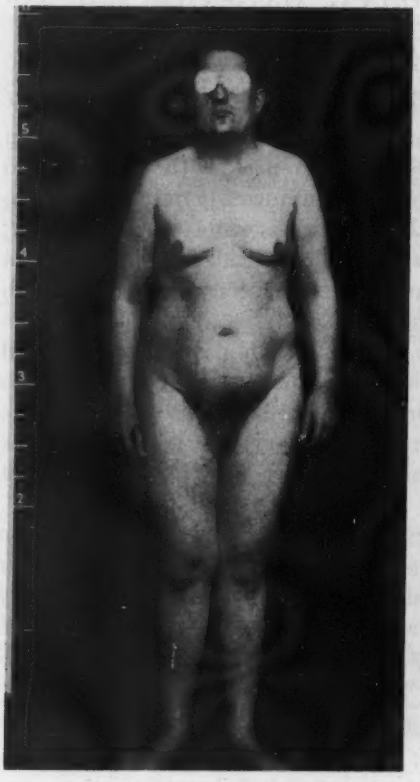

FIG. 1. Patient J. B. C., aged 26.

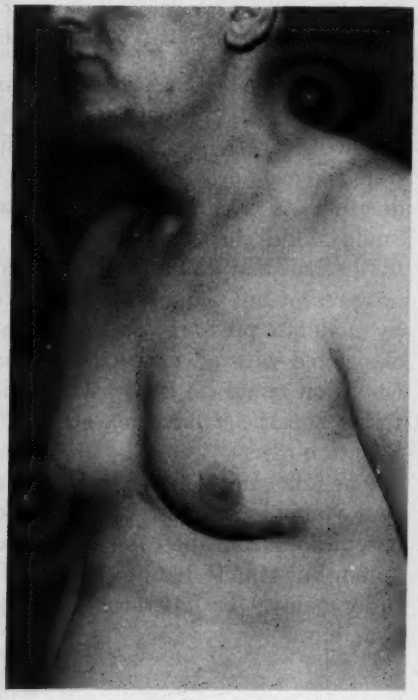

FIG. 2. Close-up photograph showing gynecomastia.

abdomen. His rather high-pitched voice and his demeanor suggested that he was not strongly masculine.

His beard was sparse and the axillary hair, as well as the hair on the arms and legs, was scanty. The pubic hair was distributed in a feminine pattern. The most striking clinical feature was the bilateral gynecomastia (Fig. 2) and the fact that the breasts contained a milky white fluid which could be expressed from the nipples. The patient demonstrated on repeated occasions that he could eject a surprisingly forceful stream of the fluid by compressing the periareolar tissue with his fingers. The penis appeared to be small but within the range of normal size, and the testes were normal in size and consist-

ency. The prostate was considered small but within the range of normal size. There were no clinical features to suggest acromegaly or Cushing's syndrome. A roentgenogram of the skull revealed a large sella turcica with erosion of the posterior clinoids (Fig. 3). Some calcification was present within the sella. Optic discs and visual fields were normal.

Initial laboratory data were: urine normal; red blood cell count, 5,240,000; hemoglobin, 92 per cent; white blood cell count, 9,050; blood sugar level, 156 mg. per 100 ml. post prandially; Wassermann and Kahn, negative; basal metabolic rate, −14 per cent; blood urea concentration, 27 mg. per 100 ml.; serum cholesterol concentration, 136 mg. per 100 ml.; and urinary 17-ketosteroids,[1] 14.5 mg. per twenty-four hours (normal 8 to 14

FIG. 3. Roentgenogram showing ballooning of the sella turcica and erosion of the clinoid processes.

mg.). Semen analysis: volume, 1 ml.; sperm count, 197,000,000 per ml.; abnormal forms, 25 per cent; and motility 75 per cent, 4 plus.

Second visit: Eleven months later the patient was re-examined. He had been receiving a diet of 1,100 calories, desiccated thyroid in a dosage of 1 grain daily, and roentgen therapy to the pituitary. Over a 36-day period the patient had received a total of 2,475 r; three months later a second series of treatments had been administered totaling 2,700 r. There was little clinical improvement; the headaches persisted, there was no lessening of lactation, no change in libido or potency, and no changes in the visual fields nor in the roentgenographic appearance of the sella turcica.

Semen analysis: volume, 1.7 ml.; sperm count: 132,250,000 per ml.; abnormal forms, 28 per cent; and approximately 80 per cent of the spermatozoa were highly motile.

[1] Urinary 17-ketosteroids were determined by the method of Bowman (4).

Third visit: The patient was next seen seven months later (one and a half years after initial examination). Since his last visit he had been receiving a diet of 1,100 calories, 1 grain of desiccated thyroid daily, and 25 mg. of testosterone propionate intramuscularly three times weekly for four months. The headaches were worse during testosterone treatment, which he stopped voluntarily. Erections had occurred about once weekly, and nocturnal emissions occasionally. There was no change in lactation and no change in visual fields.

Gonadotropin assay: the titer of FSH[2] in a 24-hour specimen of urine was found to be more than 6.6 m.u. and less than 13 m.u. Semen analysis: volume 2.0 ml.; sperm count, 75,000,000 per ml.; abnormal forms, 45 per cent; and motility 75 per cent, 4 plus.

Fourth visit: Six months later (two years after initial examination) the patient was seen again. Since his last visit, he had continued to take 1 grain of desiccated thyroid daily. The headaches had increased in frequency and lactation persisted. There were no changes in the visual fields nor in the size of the sella turcica.

Semen analysis: volume, 2.3 ml.; sperm count, 90,000,000 per ml.; abnormal forms, 86 per cent; and motility 40 per cent, 4 plus.

Fifth visit: The patient was re-examined six months later (two and a half years after his initial visit). He had been taking testosterone propionate, 25 mg. intramuscularly three times weekly for four months. The headaches had persisted but had not been severe. He had had frequent erections and occasional nocturnal emissions while taking testosterone, both of which had become less frequent since omission of this therapy. Lactation had been somewhat suppressed during the first few weeks of testosterone treatment but, despite continued treatment, it returned to its previous level and persisted.

The titer of urinary FSH at this time was found to be more than 53 m.u. and less than 105 m.u.; and 17-ketosteroids measured 10.0 mg. per twenty-four hours. Semen analysis: volume, 1.4 ml.; sperm count 72,000,000 per ml.; occasional abnormal forms; and motility 75 per cent, 3 plus.

Sixth visit: Two months later (two years and eight months after the initial examination) the patient was again seen. He had been taking 1 grain of desiccated thyroid daily. At this time he received roentgen therapy totaling 2,200 r. Clinically, the patient showed no change and no suppression of lactation was evident.

Urinary FSH was found to be more than 26 m. u. and less than 53 m. u.; 17-ketosteroids were 9.9 mg. per twenty-four hours. Semen analysis: volume 2.0 ml.; sperm count 92,000,000 per ml.; abnormal forms 20 per cent; and motility 10 per cent, 2 plus.

Estrogens in a 24-hour specimen of urine were determined by the courtesy of Dr. Joseph Jailer, using the fluorometric method. The estrogen content was estimated to be 36.5 micrograms. This was considered not to be a significant excess of estrogen excretion when compared to the estrogen excretion (15 to 28 micrograms) of a normal young man who was in Dr. Jailer's laboratory at that time.

Prolactin assays on 24-hour urine extracts were performed by the courtesy of Dr. R. W. Bates. Four samples were tested by the local crop-sac procedure at a 5-mg. dosage level, using a single injection of material. The results are expressed in terms of international units (I.U.) per gram of the powder obtained by the alcohol precipitation method; pH adjustments were not made. A urine specimen from a normal man was prepared by the same procedure, except that acetic acid was not added to the urine. Results were as follows:

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[2] Urinary FSH was assayed by the method of Klinefelter, Albright and Griswold (5).

Fraction		*Approx. wt. of fraction (mg.)*	*Prolactin, I.U./Gm.*
Patient J.B.C.	No. 3	300	6
	No. 4	250	3
	No. 5	275	3
Normal young man		402	20

It was surprising that greater values were not obtained with the patient's urine and that a relatively high value was obtained with the urine from the normal man. No ex-

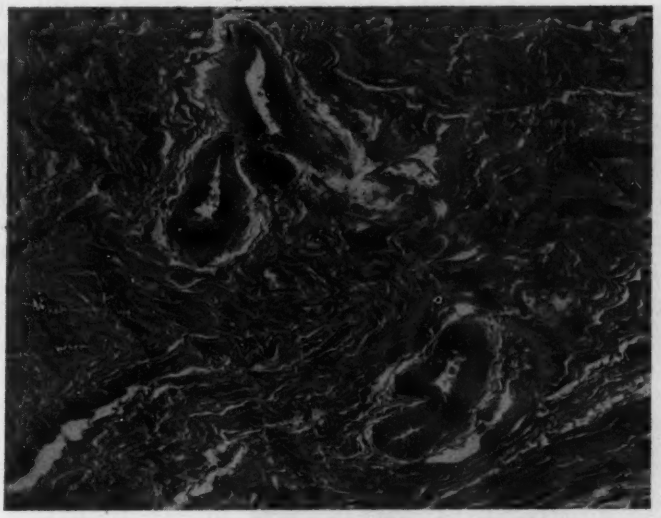

FIG. 4. Photomicrograph of tissue section from breasts.

planation is offered, except the possible effect of aging of the samples—the patient's urinary extracts were prepared some time before they were assayed, whereas the extract of the normal urine was freshly prepared.

Seventh visit: Five years after the patient's initial visit, bilateral mastectomy was performed. Dr. J. B. Hazard described the removed tissue as follows:

"The right and left specimens consisted of two large, flat, semicircular masses of bright-yellow lobulated fat. The left weighed 160 Gm. and the right 110 Gm. Multiple transverse sections made through their substance showed numerous small lobules of fat with only a rare whitish-gray thin area between the normal trabeculae. Careful search for secretory breast tissue was made but very little was found. It is likely that subareolar tissue that was spared for plastic repair, and thus escaped microscopic study, contained secretory elements. One section revealed a few ducts showing partial secretory vacuolation of epithelium" (Fig. 4).

Bilateral testicular biopsy was performed at the time of mastectomy. Microscopic examination showed (Fig. 5):

Right—Numerous average-sized seminiferous tubules were relatively widely separated in loose stroma. Gametogenesis appeared complete and active. The basement mem-

brane was moderately thickened. There were only a few interstitial cells of Leydig. The average count for both testes was a maximum of 0.1 Leydig cell per tubule, as compared to a normal count of 8 Leydig cells per tubule for a 26-year-old man (5).

Left—The findings were similar to those in the right testis, except for a normal basement membrane.

DISCUSSION

As reported by Erdheim (7), Galen was the first to use the term "gynecomastia," derived from the Greek γυνή meaning woman and μαδτός mean-

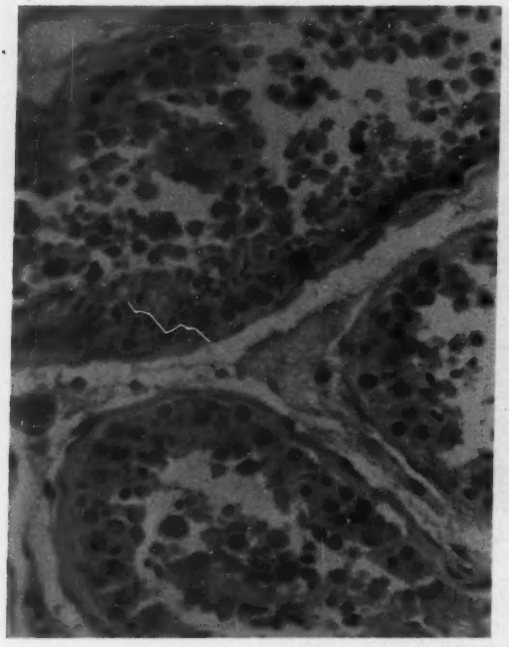

FIG. 5. Photomicrograph of section from testes biopsy specimen, showing average-sized seminiferous tubules. Gametogenesis is complete and fairly active. Basement membrane is moderately thickened. Leydig cells are few.

ing breast. It is commonly used as a clinical term to signify the enlarged male breast. Some authors use the term more specifically to indicate a certain microscopic picture of the mammary tissue. On an etymologic basis, we believe it is justifiable to apply this term in describing the form and volume of male breasts that resemble those of a female.

The breast is derived from ectodermal anlage and ordinarily remains in a quiescent state until prepuberal age. However, the "witch's milk" secreted from the breast of the newborn is well known. Its presence is a clear demonstration of the fact that the breast can respond to hormonal stimulation even in infancy. At puberty it is not uncommon to note a bud of breast tissue in the subareolar area of young males; this hypertrophy usually undergoes physiologic resolution as maturity is approached. It is

interesting that breast enlargement of a similar type has been noted in apparently normal men who are past middle age.

Gynecomastia may occur as the "simple type" and in such instances it may be unilateral. In some of these patients there are no other demonstrable abnormalities of secondary sex characteristics, and this fact, along with the fact that the gynecomastia may be only unilateral, would speak against a hormonal etiology. However, in other patients there may be a tendency to sex reversion, such as hypospadias alone or with cryptorchidism; this leaves the distinct impression of a trend toward pseudohermaphrodism.

Gynecomastia has been reported (8, 9) to accompany a large variety of conditions. It may be due to endocrine disease or to endocrine therapy; on the other hand, it may be associated with one of a number of systemic diseases in which no endocrine imbalance has been demonstrated. Furthermore, an "idiopathic" type is seen frequently, in which no evidence of underlying disease is present.

Gynecomastia is common in cases of testicular disease (9), such as chorion epithelioma or cryptorchidism. It may also be observed following testicular injury and atrophy or castration early in life. Klinefelter and associates (10) described a syndrome of gynecomastia associated with small, hard testes and aspermatogenesis without aleydigism. Heller and Nelson (11) called attention to the fact that gynecomastia in such cases seems to be a more constant feature when other signs of eunuchoidism are few or absent.

Pathologic changes in the adrenals have been known to account for gynecomastia. It is believed that feminization in such cases is likely due to the presence of increased amounts of estrogens, the source of which may be an adrenal tumor (12, 13).

Gynecomastia has been described as occurring in hyperthyroidism and in diabetes mellitus, but is by no means a frequent finding in these conditions.

Enlargement of the breasts has been reported as occurring in men who are receiving estrogen treatment for carcinoma of the prostate. A similar condition has been observed to follow the injection of androgens, chorionic gonadotropin, desoxycorticosterone acetate, or adrenocortical extract (14–16).

Gynecomastia frequently is seen in patients with hepatic disease or in states of starvation and malnutrition (17–20).

The term "lactation" has been applied in some reports as indicating various types of secretion. It would be better to limit the use of this term to the presence of a milky fluid only. In our case no analysis of this fluid was made. Roth (1) examined the milky fluid in his case and found fat globules, but no leukocytes or colostrum corpuscles. The fluid was alkaline and

contained casein, fats, lipoids, lactose, as well as 8.5 per cent sodium chloride.

In our case the roentgenographic evidence of a ballooned sella turcica, the distinctly thinned clinoid processes, and the symptom of persistent headache were sufficient indications of the presence of pituitary tumor. There was no evidence of pressure on the optic chiasm, and serial studies showed that the visual fields remained normal. Periodic roentgen study of the sella turcica yielded no evidence that the tumor had enlarged.

The three 17-ketosteroid values were normal. This does not exclude gonadal failure, but at least does not support the suspicion of its presence; neither does it indicate adrenal hyperactivity, though here, too, it would not relate to overproduction of estrogens. Tubular testicular failure obviously was not present. The semen was normal, repeatedly; the mild abnormality in some of the specimens was likely the result of testosterone therapy. The testes biopsies revealed normal tissue, except for some thickening of the basement membrane and a reduction in the number of Leydig cells; and there was no excess of urinary gonadotropins. These findings clearly exclude Klinefelter's syndrome. The amount of estrogen excreted was not considered to be excessive, when compared to that of the normal young man. The evidence seems to point to the pituitary tumor as the cause of the gynecomastia with persistent lactation. It seems possible that a selective hypersecretion of some tropic hormone may have been involved, but this could not be substantiated by the prolactin assays. Roentgen therapy had no noticeable effects; in view of recent reports, this negative result may have been due to inadequate roentgen treatment (21).

On one occasion testosterone propionate in a dosage of 25 mg. intramuscularly three times weekly for four months resulted in no lessening of the headaches or the lactation. The patient's libidio and potency were not increased during this period of treatment. When similar therapy was carried out for a comparable length of time one year later, the amount of lactation was thought to be suppressed only during the first weeks of treatment; then it returned to its preceding level in spite of continued therapy. Whether or not there was any actual pituitary inhibition is questionable.

SUMMARY

A case of gynecomastia with persistent lactation in a 26-year-old man is reported. In addition to lactation there were two outstanding features of interest, namely, a pituitary tumor, and normal gametogenesis. No abnormal patterns of estrogen and prolactin excretion could be demonstrated.

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SPONTANEOUS REMISSION IN A CASE OF CUSHING'S SYNDROME

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CUSHING's syndrome is usually caused by hyperplasia or carcinoma of the adrenal cortex; less frequently it is associated with a basophil tumor of the pituitary, with or without adrenal hyperplasia; exceptionally it is observed in the presence of an ovarian or a thymic tumor; and in rare instances autopsy of typical cases of Cushing's syndrome reveals no organic lesion in the endocrine glands. Irrespective of its original cause, the evolution of all known cases has followed a progressive course which could only be delayed by means of pituitary irradiation, ample adrenalectomy, or death of the patient due to some intercurrent affection.

Spontaneous remission in cases of Cushing's syndrome with well defined somatic alterations is mentioned only exceptionally in the literature (1–4), and for this reason we consider as most interesting the description of a typical case, including adrenal hyperplasia, in which there was spontaneous evolution to complete cure.

CASE REPORT

(No. 18,487). The patient was an unmarried woman, 21 years old, born in the Province of Catamarca, Argentina. The family history was non-contributory. When she was 11 years old, she had a fever for two months which resembled that of brucellosis. When she was 16 she had measles and mumps; at 20 she had German measles. Menses began at the age of 14½; the interval was twenty-five days and the bleeding lasted for three days.

She was admitted to the Clinical Ward on July 2, 1953, suffering from a disease characterized by the following symptoms: two months of amenorrhea after two years of irregular menses with periods of oligomenorrhea associated with hypomenorrhea and hypermenorrhea; headache with elective retro-ocular localization during the preceding year; and an increase in weight from 62 Kg. to 69 Kg. during the same period. She had noticed that obesity was most prominent in the face and was associated with substantial changes in features and expression. There had been a gradual increase in facial and body hair, and marked shedding of scalp hair. During the previous six months she had noticed atrophic striae in different parts of the body, which increased gradually in number and

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size. During the previous two weeks she had felt pain in the interior thorax, spreading forward in the form of a belt. She had no other complaint.

Physical examination revealed obesity of moderate degree. Her weight was 69 Kg. and height, 158 cm. Adipose tissue had accumulated in the face, neck (especially in the posterior part) and thorax. The abdomen was moderately prominent and the gluteal regions definitely flattened. The arms and legs appeared normal. The general body configuration was of the buffalo type (Fig. 1A). The features showed the characteristic moon

FIG. 1. **A.** The patient at the peak of Cushing's syndrome. **B.** One year later.

shape, with drooping of the corners of the mouth and an increase in adipose padding of the cheeks (Fig. 2A). The eyes, nose and mouth were normal. There was no pigmentation of the buccal mucous membrane. The skin was brown, plethoric and seborrheic. There were extensive violet-red striae (more than 2 cm. in width) with telangiectasia in the external surface of the upper arms, axillae, hands, anterior and lateral abdominal walls, iliac and trochanteric regions, and antero-medial surfaces of both thighs. There was a marked increase in facial hair, especially on the cheeks and upper lip; body hair was abundant in the dorsal region and on the shoulders and arms; axillary and pubic hair was very thick,

and the latter had a masculine distribution. The scalp hair receded over the temples and, although it remained abundant, shedding was appreciable. The muscular system was normal, as was the skeletal system, except for dorsal kyphosis. The thyroid gland was normal in size. The mammary glands were large, with deeply pigmented areolae; the mammary tissue was of normal consistency. A study of the cardiovascular system revealed normal heart sounds with a rate of 96, an arterial blood pressure of 152/100 and a heart of normal size by roentgenography. The respiratory system was normal. Abdominal findings were negative. Gynecologic examination showed no abnormalities;

FIG. 2. **A.** Facial features characteristic of Cushing's syndrome during the climax of the disease. **B.** Facial features have returned to normal and to her original appearance (*cf.* Fig. 4).

the clitoris was of normal size. Results of examination of the nervous system were negative.

Roentgenograms of the sella turcica showed normal characteristics; those of the spine showed diffuse osteoporosis of moderate degree. Adrenal radiography, after the injection of air by the precoccygeal route, revealed a bilateral increase to double the normal size; the increase was diffuse and symmetrical (Fig. 3). Pelvipneumography showed the ovaries and uterus to be of normal dimensions.

The patient retained a good appetite, having reduced voluntarily the amount of carbohydrates and sodium chloride consumed, for the purpose of losing weight. She had polydipsia and polyuria of moderate degree.

Laboratory data: RBC 4,620,000; hemoglobin 14.2 Gm. per cent; WBC 7,000 with neutrophils 75 per cent, eosinophils 22 per cent, lymphocytes 18 per cent and monocytes 5 per cent. The level of blood urea was 30 mg. per 100 ml., cholesterol 286 mg., chloride 400 mg., sodium 344 mg., potassium 20 mg., total calcium 8.3 mg., phosphorus 3.6 mg.,

and alkaline phosphatase 2.6 Bodansky units per 100 ml. The urine was normal, without albumin or glucose and with a specific gravity of 1.015. Three successive glucose tolerance tests, with 100 grams of glucose orally, yielded the following results:

	Blood Sugar Level (mg./100 ml.)			
	0	*60 min.*	*120 min.*	*180 min.*
July 8, 1953	70	95	90	80
Aug. 3, 1953	80	80	90	80
Nov. 2, 1953	80	115	100	85

An insulin tolerance curve with 0.1 unit per Kg. subcutaneously was as follows:

0	*15 min.*	*30 min.*	*60 min.*	*80 min.*	*120 min.*	*180 min.*
85	45	40	60	70	75	80

FIG. 3. Adrenal radiogram after injection of air by precoccygeal route, showing increase in the shadow of both adrenals.

Urinary 17-ketosteroids were 24.2 mg. per twenty-four hours. 11-Oxycorticosteroids on both July 3 and July 16, 1953 were 1.2 mg. per twenty-four hours. The urinary gonadotropin titer was less than 48 mouse units per twenty-four hours. Thorn tests with 25 mg. of ACTH resulted in an eosinophil count of 223 per cu. mm. before, and 190 per cu. mm. after four hours—a drop of 14.8 per cent.

Clinical course. No treatment was instituted except for a restriction in caloric intake.

In August 1953 menstruation, although scanty, reappeared and continued monthly. In December her weight was 66.4 Kg., with a general decrease in adipose tissue and a decrease in the symptoms typical of Cushing's syndrome. At that time, methyltestosterone in a dosage of 10 mg. daily was given for a few days. In January 1954, all pathologic somatic characteristics had become much less pronounced, the cutaneous striae were becoming pale and the skin was more normal in appearance. In April the improvement was marked. The patient's weight was 61.9 Kg., the blood pressure was 110/75, and there was a great change in her facial expression. In July 1954 her features and somatic configuration were completely normal (Figs. 1B and 2B) in spite of an increase

in weight to 65.5 Kg; the cutaneous striae were completely pale and the blood pressure was 90/60.

Determinations of urinary 17-ketosteroids gave the following results: August 28, 1953, 14.9 mg.; November 3, 1953, 9.4 mg.; and May 10, 1954, 12.5 mg. per twenty-four hours. The 11-oxysteroid values were: November 3, 1953, 0.95 mg.; and May 10, 1954, 1.04 mg. per twenty-four hours.

After suffering from Cushing's syndrome for almost a year, the patient had returned to her physical status prior to the disease (Fig. 4) and was completely free of symptoms. Menstruation was normal.

FIG. 4. The patient two years before the onset of the disease.

COMMENT

The patient in question was undoubtedly a case of Cushing's syndrome. Her appearance was characteristic. The facial expression, distribution of obesity, active skin striae, somatic conformation, amenorrhea, hypertension, increase in urinary 17-ketosteroids and accentuated increase of the adrenal area by radiologic examination were all in favor of the diagnosis.

A year before she was examined, the patient had a normal appearance as demonstrated by photographs. A year after the diagnosis of Cushing's syndrome was made, her appearance had returned to normal as shown by comparing photographs. In spite of the fact that she retained the weight she had put on during the disease, her face no longer presented any trace of abnormality. Blood pressure and urinary 17-ketosteroids had both decreased to normal levels. Unfortunately the patient could not be persuaded to undergo a second radiologic examination of the adrenals.

There is one plausible interpretation of the pathogenesis in this case, namely, a functional alteration (probably hypothalamohypophyseal in origin) with an endogenous increase of corticotropin causing stimulation of the adrenal cortex and consequent hyperplasia, hyperfunction and the development of Cushing's syndrome. Experimentally, it has been shown that the hypothalamus can bring about hyperfunction of the anterior pituitary with increased elaboration of corticotropin (5), but there is no proof that this can happen in the human being. The present case could be considered as a possible example.

However, there are other explanations which cannot be discarded, such as a primary hyperplasia of the adrenals, or an increase in adrenal activity with congenital hyperplasia. The possibility of a lesion involving the hypothalamic nuclei, of the type observed experimentally by Heinbecker (6), could not be demonstrated in the present case.

Whatever the cause of the adrenal hyperactivity—a hypersecretion of pituitary corticotropin (as we are inclined to believe), an autonomous adrenal hyperfunction, or an adrenal hyperplasia which became temporarily functional—we have no explanation for the spontaneous disappearance.

SUMMARY

In a 21-year-old woman, previously in good health, typical Cushing's syndrome developed in the course of a year, with amenorrhea, characteristic facies and somatic conformation, atrophic cutaneous striae, hypertension, and an increase in urinary 17-ketosteroids. Adrenal radiography showed an increase in the size of both glands. A year after the diagnosis was made, there was no trace of the disease and the patient had returned to her original appearance. The cause of this condition could not be determined. Possible interpretations are discussed.

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FEMALE PSEUDOHERMAPHRODISM WITH PENILE URETHRA, MASQUERADING AS PRECOCIOUS PUBERTY AND CRYPTORCHIDISM.

CASE REPORT

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INTRODUCTION

FEMALE pseudohermaphrodism is characterized by varying degrees of masculinization of wolffian and müllerian derivatives, present at birth, in a gonadal female. Subsequently, other somatic and secondary sexual characteristics deviate toward the masculine and may appear precociously. Typically, the female pseudohermaphrodite at birth has an enlarged *nonperforate phallus* with a single opening (urogenital sinus) at its base, large, empty scrotum-like labia majora and absent or vestigial labia minora. The urethra and a short vagina open into the urogenital sinus. The cervix, uterus, tubes and ovaries may be fully or incompletely developed. Prostatic elements may be present. Such a combination of virilization and relatively obvious developmental abnormalities of the external genitalia will, on clinical grounds, indicate the necessity of differentiating a *female* pseudohermaphrodite from a hypospadiac male with sexual precocity and cryptorchidism.

The extreme rarity of this aberration (1, 2) and the confusion in diagnosis, exemplified by the following case in which the prenatal masculinization of the external genitalia with a penile urethra was so complete that it entirely obscured the true condition, seems to justify a detailed report.

CLINICAL FEATURES

Patient J. H. M.[1] (UCH-OPD 239660), was born July 28, 1932, and was first admitted to the University of California Hospital at the age of 22 months because of "sexual precocity and cryptorchidism." The father (aged 42) and the mother (aged 37)

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[1] Photographs of this patient appeared in the book by Lawson Wilkins, "The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence," Springfield, Illinois, Charles C Thomas, Publisher, 1950, pp. 240, 264.

were in good health and not known to be consanguineous. Two brothers, aged 5 and 6½ years, respectively, and one sister, aged 13 years, had developed normally. There was no family history of similar or related abnormalities.

The patient weighed 8¼ pounds at birth after an uncomplicated full-term pregnancy and delivery. Early development was considered normal. The child was active and strong. Past illnesses included mild and uncomplicated measles, pertussis and chickenpox. There was no history suggestive of addisonian adrenocortical insufficiency. It was not until the patient was 15 months old that an enlarging phallus attracted attention to the genitalia.

FIG. 1. Patient J. H. M., a female, aged 22 months. Note boyish stature.

The child was first hospitalized at the University of California Hospital at the age of 22 months (Fig. 1), and weighed 32½ pounds (normal, 22.9 to 28.5) and was 37 inches tall (normal, 32.2 to 34.6) at that time. The blood pressure was 104/60. There were sixteen well-formed teeth and no abnormal pigmentation of the skin or mucous membranes. The degree of development of the phallus was striking (Fig. 2). It measured 4 cm. in length and 4 cm. in circumference, became erect during examination, and in all respects resembled a normal penis. Some rather sparse, long coarse hairs were noticeable. The scrotum, although empty, appeared normal otherwise. There was no perineal dimple. Nothing was felt in the inguinal canals; the external inguinal rings were large. On rectal examination, tissue in the area of the prostate was palpated and what was thought to be prostatic fluid was expressed.

Results of routine blood and urine examinations, phenolsulfonphthalein excretion

tests and glucose tolerance tests were normal. The level of serum sodium was 311 mg. per 100 ml. (135 mEq. per liter); potassium, 18.2 mg. per 100 ml. (4.7 mEq. per liter); and chloride, 308.9 mg. per 100 ml. (88 mEq. per liter). On two occasions the 24-hour urinary excretion of sodium was 0.921 and 0.863 gram. Intravenous and retrograde urograms showed no abnormalities. The epiphyseal age was estimated at between 8 to 10 years by roentgenogram, an acceleration of fully 6 to 8 years.

The diagnosis was considered to be "sexual precocity and cryptorchidism abdominalis." The adrenals were explored surgically through the nephrectomy approach and appeared grossly normal. A small biopsy specimen was obtained from the right adrenal and on microscopic examination was reported as "adrenal cortical tissue showing no pathological changes." The postoperative course was uneventful, and the child was discharged from the hospital with instructions to return later for orchidoplasty.

FIG. 2. Close-up of external genitalia. Note fairly large "penis" for age (even if the patient had been a boy) and long, sparse pubic hairs.

During the two months preceding the final hospitalization, the child was well except for recurrent priapism. It was not until abdominal exploration for orchidoplasty at the time of the second hospital admission that the true gonadal status was discovered. Neither testes nor cords were found, but instead well-formed fallopian tubes with fimbriated ends, ovaries and a small, normally formed uterus. Postoperatively, the child became febrile, showed pulmonary rhonchi, tachycardia, tachypnea and cyanosis, and died in respiratory failure on the first postoperative day.

POSTMORTEM EXAMINATION

The lungs were congested and most small bronchioles of the lower lobes oozed thin purulent material. The heart weighed 80 grams; the right ventricle was markedly dilated; the foramen ovale was closed, and the ductus arteriosus was patent. There was generalized lymphadenopathy. The thymus was enlarged, weighing 40 grams, but was essentially normal on microscopic examination. The pituitary weighed 0.35 gram.

No testes were found; a normal-appearing uterus, tubes, ovaries and greatly enlarged

convoluted adrenals were present instead (Fig. 3). The adrenals were similar in size and together weighed 30 grams (Fig. 4). There were a few cortical adenomata. Microscopic examination showed a marked cortical hyperplasia, due partially to an increase in cells of the fascicular layer but chiefly to an increase in reticular cells (Fig. 5). The hyperplastic reticular cells had invaded the adrenal medulla to such a degree that only disconnected smalls island of medullary tissue were found scattered throughout the reticular cells.

The ovaries contained numerous ova and well-developed follicles. The anatomy of

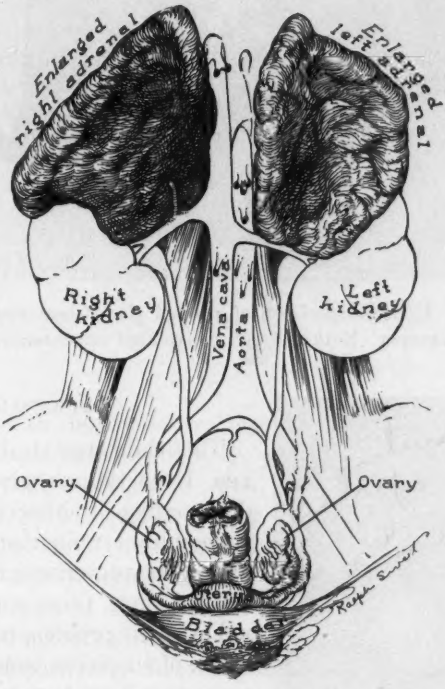

FIG. 3. Drawing made at autopsy. Note large adrenals, together weighing 30 Gm. (normal weight together in adults, 11 Gm.). Note also normal infantile uterus, tubes and ovaries.

the urogenital tract is depicted diagramatically in Figure 6. Distal to the bladder, the urethra passed through a firm nodule of tissue which resembled a prostate; however, this structure consisted of a thick outer layer of circular striated muscle and a thin inner layer of smooth muscle fibers with no trace of prostatic tissue. Centrally located in this mass of tissue was a small duct-like opening which led posteriorly to a well-formed vagina, cervix and uterus. Distal to this nodule, the urethra continued through the entire length of a perfectly formed "penis."

The final anatomic diagnoses were female pseudohermaphrodism, bilateral adrenal cortical hyperplasia, patent ductus arteriosus, and bronchiolitis.

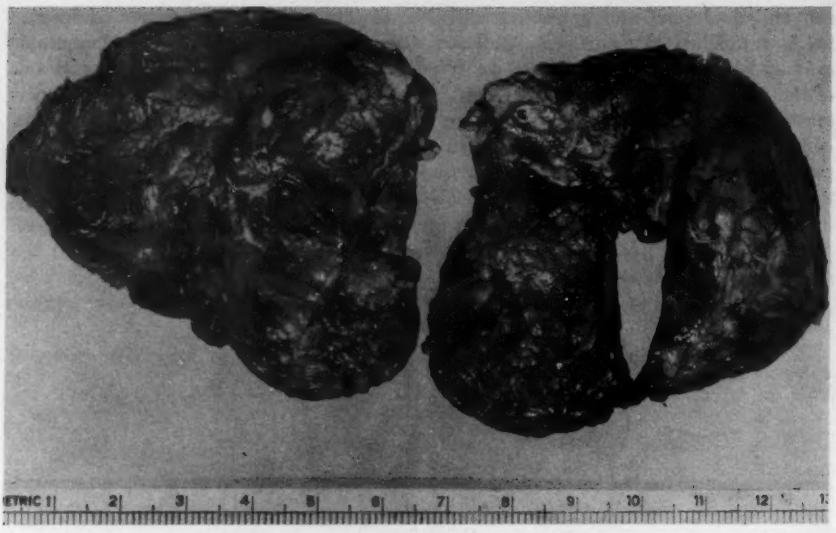

FIG. 4. Gross specimen of adrenal glands removed at autopsy. Note size and convoluted appearance.

FIG. 5. Photomicrograph of hyperplastic adrenal. Hyperplasia chiefly involved reticular zone. Reticular cells invaded medulla, leaving only islets of medullary cells.

DISCUSSION

This child was studied twenty years ago. Even though advances in the understanding of endocrine problems and concomitant refinements in laboratory diagnostic procedures have been accomplished since, there would be no less of a diagnostic problem today. Only a high index of suspicion would lead to definitive procedures to establish the diagnosis of female pseudohermaphrodism in the face of clinical evidence pointing to a more probable diagnosis of the adrenogenital syndrome with cryptorchidism. Admixtures of precociously and incompletely developed external genitalia, as well as cryptorchidism, do occur in the adrenogenital syndrome in gonadal males (3–5).

Definitive diagnosis is imperative in

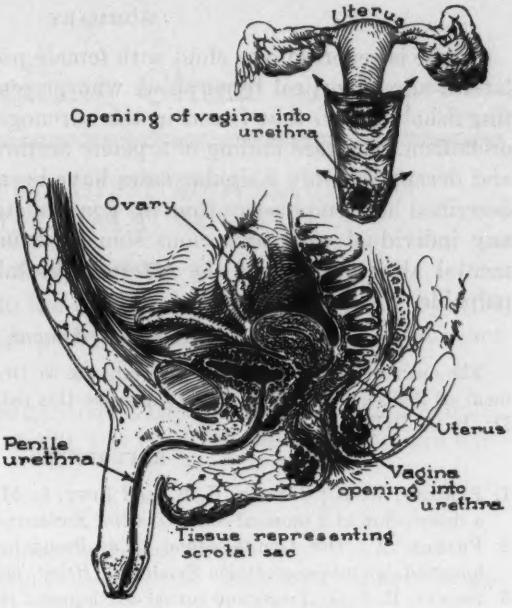

FIG. 6. Dissection of female internal genitalia. Note well-formed cervix opening into vagina which connects with the posterior urethra. Sagittal section shows fairly large penis-like structure with urethra extending completely through and to end of phallus, exactly duplicating penis of a small boy.

these tragic cases so that measures may be taken, insofar as possible, to correct the developmental errors early, to diminish the progressive somatic masculinization that is certain to occur, and to guard against the adrenocortical insufficiency with which it is so frequently associated (6).

The case of this child emphasizes that the question of gonadal status should be raised in any individual with precocious somato-sexual developmental abnormalities of the external genitalia, in whom testes are not palpable. Symptoms of adrenocortical insufficiency, other instances of somato-sexual abnormality in the same sibship, or the history that another sibling died of "pyloric stenosis," "projectile vomiting," or "starvation" further emphasize the need of complete investigation, for these are symptoms suggestive of adrenal insufficiency of the salt-controlling hormone in the newborn.

The definitive diagnosis of female pseudohermaphrodism has depended upon exploratory laparotomy and biopsy of the gonads. Recently, the newly developed technique of detection of chromosomal sex in hermaphrodites on the basis of skin biopsy finding (7) offers promise of a relatively simple and safe means of determining the gonadal status when there is evidence that the somatosexual abnormality is associated with the congenital adrenal syndrome (8).

SUMMARY

A case is reported of a child with female pseudohermaphrodism and bilateral adrenocortical hyperplasia who presented a clinical picture indistinguishable from that found in the adrenogenital syndrome with cryptorchidism. The rare finding of a penile urethra was especially noteworthy and deceptive. Only 5 similar cases have been reported to date. The case described here emphasizes that the gonadal status should be questioned in any individual with precocious somato-sexual development or developmental abnormalities of the external genitalia in whom testes are not palpable.

Acknowledgment

The authors herewith record their gratitude to Dr. William Deamer of the Department of Pediatrics for permission to describe this patient, originally under the care of Dr. W. A. Reilly.

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Teaching Clinic

HYPOPARATHYROIDISM AS A CAUSE OF OSTEOMALACIA.*

CASE HISTORY

On a hot June 28th in 1940 the patient, Miss E.L. (#254647), aged 31 came to my office complaining, among many other things, of incontinence of urine.

Her appearance was most suggestive of Graves' disease—exophthalmos, tremor, perspiration, a pulse of 120, a B.P. of 190/90, weakness, and a respiratory wheeze.

Past history

The following items seemed important in the past history:

1) March 1935—removal of a corpus-luteum cyst for menorrhagia and metrorrhagia.
2) October 1935—supravaginal hysterectomy for the same symptoms.
3) February 1936—subtotal thyroidectomy; pathologic report, "normal thyroid."
4) October 1936—thyroid operation repeated; thymus enlarged; some thyroiditis; preoperative symptoms remained; those of "laryngitis" added.
5) June 1937—third thyroid operation.
6) August 1937—exploration of left adrenal followed by left splanchnicectomy (L. I and II); incontinence of urine and left hemianaesthesia both added to list of symptoms.
7) October 1938—right splanchnicectomy.

Diagnoses

Dr. F. A.: Recurrent Graves' Disease. Medical Service: Anxiety Neurosis, Hysteria, Asthma.

Contribution of Psychiatric Service

The patient was transferred to the Psychiatric Service on July 11, 1940. Her general condition improved but incontinence and other specific symptoms remained unchanged. The cystometrogram was normal. On January 3, 1941, one hundred and eighty-nine days

* Read by title at the Sixty-Seventh Annual Meeting of the Association of American Physicians, Atlantic City, New Jersey, May 5, 1954.

Read at the Third Pan American Endocrinology Congress, Santiago, Chile, November, 1954.

Supported in part by grants-in-aid from the United States Public Health Service, the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, the Rockefeller Foundation, and Ayerst, McKenna & Harrison, Ltd.

after admission, she was considered ready for discharge. The discharge diagnosis of the Psychiatric Service was to have been Multiple Sclerosis.

Contribution of Dental Department—Act of God

The night before she was to have been discharged a toothache developed. A dental x-ray examination showed generalized demineralization of the mandible. A diagnosis of hyperparathyroidism was suggested; accordingly the following three laboratory determinations were carried out:

Serum calcium	5.9 mg. per 100 cc.! (low)
Serum phosphorus	5.1 mg. per 100 cc.! (high)
Serum alk. phosphatase	8.2 B.U. per 100 cc.! (high)

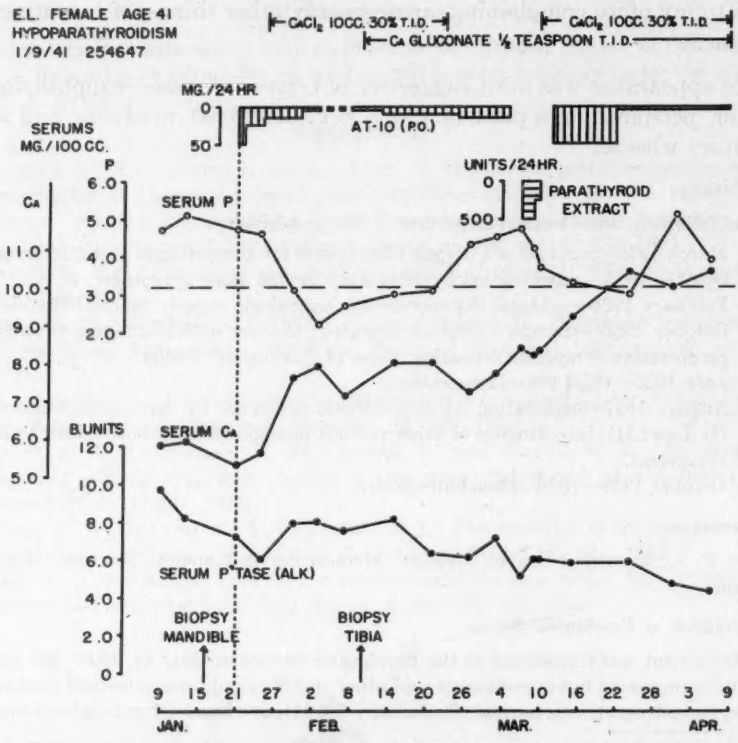

FIG. 1. Serum calcium, phosphorus, and alkaline phosphatase values in relation to treatment of hypoparathyroidism. Note the high serum phosphorus and the low serum calcium level before treatment, indicative of hypoparathyroidism; note the high serum alkaline phosphatase level, indicative of osteomalacia; note the excellent response to therapy in respect to all 3 indices; note that parathyroid extract caused marked lowering of the serum phosphorus level, which is strong evidence against pseudohypoparathyroidism; finally note that the biopsy specimen of the mandible was taken before treatment, whereas the biopsy specimen of the tibia was taken twenty days after the onset of treatment.

Came the dawn!

The diagnosis was not hyperparathyroidism, but postoperative hypoparathyroidism! The wheeze was a manifestation partly of recurrent nerve injury, or of tetany, or both! Bladder symptoms improved markedly following appropriate treatment for hypoparathyroidism. Disordered blood levels of calcium, phosphorus and phosphatase responded to specific therapy (see Fig. 1).

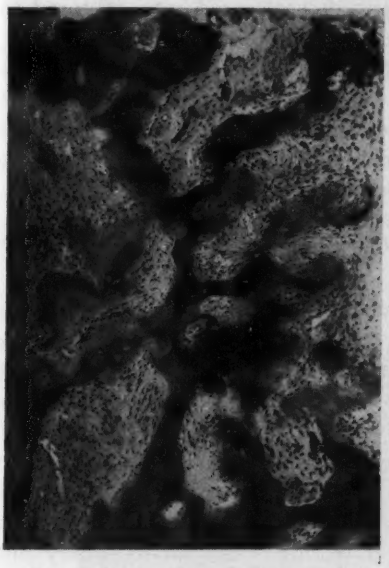

FIG. 2. Section from biopsy specimen of the mandible before treatment. Note that almost all trabecular surfaces are covered with wide osteoid seams and osteoblasts. (The author is indebted to Dr. Granville A. Bennett for this and the next biopsy specimen.)

FIG. 3. Section from biopsy specimen of the tibia. Remember that this tissue was obtained twenty days after onset of therapy and at a time when the blood indices had returned almost to normal. Note that there is no evidence of osteomalacia. It is appreciated that this biopsy specimen comes from a very different source than that of the first specimen.

Why the high concentration of phosphatase?

One does not usually anticipate a high serum phosphatase level in hypoparathyroidism. It suggests osteitis fibrosa generalisata, osteomalacia, and one or two other things. A biopsy of the mandible at the time of the extraction was interpreted by Dr. Granville A. Bennett as "consistent with osteomalacia" (Fig. 2).

Digression on effect of hypoparathyroidism on rats' teeth

Does hypoparathyroidism beget osteomalacia? The answer is "yes." The conditions must be just right, however.

Dentinoid is to rat's dentine what osteoid is to human endosteal bone. The dentine of the incisor teeth of rodents is worn down at the biting surface and replaced, layer by layer, by the odontoblasts (counterpart to osteoblasts) from the pulp cavity. Professor J. Erdheim in 1906 (1) showed that, from the time the parathyroids were removed from rats, the newly laid down dentinoid was no longer calcified. Odontomalacia (if there is any such word) developed. Erdheim and Albright in 1930 (2) showed that parathyroid extract, administered to parathyroidectomized rats, restored to normal this *a*-calcification of the dentinoid (Fig. 3). Finally, Erdheim in 1911 (3) showed that parathyroidectomy in rats had an osteomalacic as well as an odontomalacic effect; this was especially true as regards the calcification of the newly formed calluses at the sites where bone fractures were induced. We might therefore conclude, at least tentatively, that the osteomalacia was due in some mysterious way to the hypoparathyroidism.

How reconcile a-calcification in hypoparathyroidism with de-calcification in hyperparathyroidism?

It would seem at first sight that hypoparathyroidism and hyperparathyroidism should have opposite effects on bone. That they do not will take a little explaining. In the first place it must be remembered that bone formation is not the opposite of bone destruction. Hyperparathyroidism affects bone resorption; hypoparathyroidism affects bone formation. Leave aside for the time being all thought of units. Grant for the moment that some solubility product plays a major rôle in governing the concentration of calcium and phosphate ions in body fluids. Since we do not know know which salt of calcium and phosphate this is, nor all the many side reactions which tend to modify the ion product in question (*e.g.*, adsorption of calcium ions to various proteins, modification of phosphate concentration by various enzymes, phosphatase and phosphorylase, or complex ions), let us take the concentration of serum calcium in milligrams and the serum phosphorus in milligrams and act as if their product were a first approximation of the ion product which has a controlling effect on the level of calcium and phosphate in body fluids.

So far so good!

Digression on bone logistics

Bone is composed of two types of surface—one where bone is being resorbed, and one where it is being formed. Both of these processes go on at the same time. Where bone is being resorbed one sees osteoclasts. It is my belief that body fluids are under-saturated with respect to the solubility product of calcium phosphate and that calcium and phosphate ions are constantly being resorbed according to physical-chemical laws, at bone-resorbing surfaces. The osteoclasts probably are foreign-body giant cells.

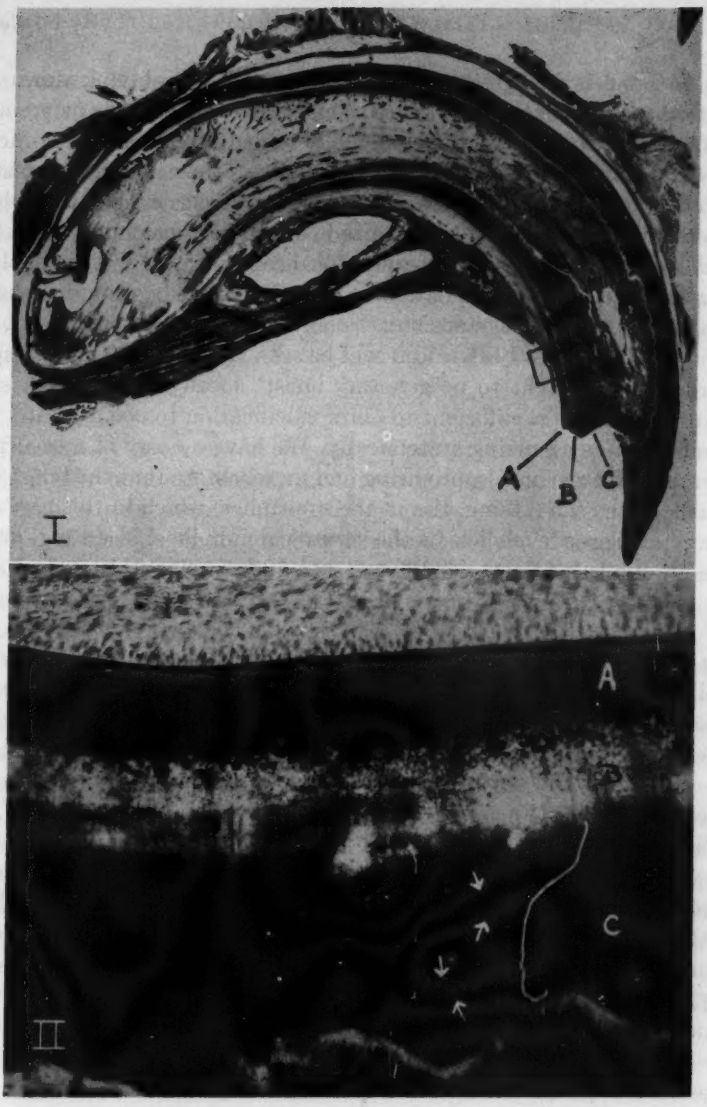

FIG. 4. Effect of parathyroidectomy and of parathyroid extract on calcification of the dentine of a rat's tooth.

I. Sagittal section through tooth: **A.** Band of calcified dentine laid down before parathyroidectomy. **B.** Band of uncalcified dentine laid down during the period of parathyroid hormone insufficiency. **C.** Band of calcified dentine laid down during period pf parathyroid hormone injection.

II. Higher magnification of boxed-off area in **I.** Note two faint lines (see arrows) of uncalcified dentine representing two Sundays when rats did not receive injections. (Erdheim, J., and Albright, F., *see* ref. 2.)

(Reproduced with permission from Transactions of the Eighth Conference on Metabolic Aspects of Convalescence. New York, Josiah Macy, Jr. Foundation, 1945.)

Where bone is being formed, one sees osteoblasts laying down bone matrix which later becomes calcified. This suggests that there may be some local factor at work, possibly the much heralded "local-calcifying-factor," at the exact site of bone formation, which allows the calcium phosphate to precipitate into the bone when right around the corner it is coming out. A priori this factor might be a localized collection of calcium ions, a localized collection of phosphate ions, or a localized rise in pH. It probably is a localized collection of phosphate ions brought about by the phosphatase-phosphorylase enzyme system.

From what has just been said it will be seen that the calcium-phosphate ion product is thought to be given a "boost" locally at the exact site of calcification, which is sufficient to allow calcification to occur locally when decalcification is occurring systemically. We have no way of measuring it, but let us again do some improvising and merely state that the boost is due to (shall we say?) a 3.0-mg. rise in the multiplier, which in this case is the serum phorphorus level. Tus, if the serum calcium level be 10 mg. and the serum phosphorus 4.0 mg. per 100 cc., then $10 \times 4 = 40$ and $10 \times (4+3) = 70$.

Effect of parathyroid hormone on bone formation

Let us now pull another number out of the hat and assume that the solubility product of calcium phosphate is 55. Then it will be seen that the ion product of 40 for normal serum before the boost is undersaturated with respect to bone-resorbing surfaces, whereas the ion product of 70 for normal serum after the boost is oversaturated with respect to bone-forming surfaces.

A little simple arithmetic will show that the size of the boost resulting from the local-calcifying-factor depends a great deal on the ratio of the phosphorus to the calcium (of the multiplier to the multiplicand). It was seen that a boost of 3 mg. applied to the phosphorus level when the serum calcium and phosphorus levels are normal (10 and 4 mg. per 100 cc. respectively) raises the product from 40 to 70. Let us see what happens to the product when the serum calcium and phosphorus values are those seen in hypo- and hyperparathyroidism.

The following equations are instructive:

Calcium		*Phosphorus*		*Ion Product*	
10	×	4	=	40:	in normal serum
10	×	(4+3)	=	70:	same plus boost
4	×	10	=	40:	in hypoparathyroidism
4	×	(10+3)	=	52:	same plus boost
20	×	2	=	40:	in hyperparathyroidism
20	×	(2+3)	=	100:	same plus boost

It will be seen that as a result of the boost the ion product rose 30 points

in the normal serum, but only 12 points in the hypoparathyroid state—not enough to reach the saturation point of 55. In the hyperparathyroid state, on the other hand, the product rose 60 points, thus exceeding the normal by 30 points and the hypoparathyroid by 48 points. It is obvious that the phosphatase-phosphorylase enzyme system, in spite of delivering the same boost, is inefficient in the presence of high phosphate levels.

It is also obvious that the possibility of osteomalacia developing in hypoparathyroidism depends on whether the boost succeeds in raising the ion product above the solubility product of calcium phosphate.

Either it does or it doesn't. In the case of Patient E.L. it didn't.

SUMMARY

1. The history is presented of a case of postoperative hypoparathyroidism which, for no good reason, remained undiagnosed for almost a year.

2. The first clue which led to the diagnosis (there were many which should have) was the finding of demineralized bone.

3. Analysis of the blood showed a low serum calcium and a high serum phosphorus level, and the diagnosis of hypoparathyroidism was apparent. The patient responded well to treatment for hypoparathyroidism.

4. Bone biopsy showed osteomalacia and, in support, the serum alkaline phosphatase level was constantly high.

5. To reconcile osteomalacia resulting from hypoparathyroidism, attention has been directed to the *a*-calcification of rats' teeth and bones following parathyroidectomy, as demonstrated by Erdheim.

6. A theory is presented to explain the mechanism of the *a*-calcification in hypoparathyroidism. It is pointed out that the localized increase of phosphate ions at the the site of calcification resulting from the action of the phosphatase-phosphorylase enzyme system is very ineffective in the presence of a high serum level of phosphate ions such as occurs in hypoparathyroidism.

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Letter to the Editor

ALDOSTERONE EXCRETION IN PREGNANCY*

To the Editor:

Earlier studies on the excretion of aldosterone in normal pregnancy (1, 2, 3) showed that this hormone was only slightly increased above the range found in the non-pregnant woman. The fraction assayed in these studies for sodium-retaining activity contained the crude neutral material extracted immediately from acidified urine. It is now recognized that the greater part of aldosterone present in urine is in a conjugated form. This can be released by hydrolysis with β-glucuronidase (4) or by allowing the acidified urine to stand at room temperature for twenty-four to forty-eight hours (5).

In the present study we have compared the two methods of hydrolysis and have measured the excretion of the free and conjugated fractions of aldosterone in pregnancy urine. The free fraction was obtained by first extracting the urine with chloroform, the glucuronide fraction after incubation of the urine with β-glucuronidase for forty hours, and the acid-hydrolyzed fraction after the remaining urine was acidified to pH 1.5 and allowed to stand at room temperature for twenty-four hours. All three extracts were purified by paper chromatography using the Bush B 5 system (6). Only that fraction running between hydrocortisone and cortisone was used for the bioassay.

The following are the values obtained for the aldosterone content of the various urinary fractions at different periods of pregnancy:

Period of pregnancy	*Aldosterone (μg.)*		
	Free	*Glucuronide*	*Acid hydrolysis*
3¾	2.0	10.3	12.5
4¼	1.4	4.1	19.4
8	1.8	8.4	14.0
8	5.0	12.0	4.0
9	1.3	2.5	20.9

These results showed that there was very little increase in the excretion of free aldosterone throughout pregnancy. The aldosterone content of the glucuronide and the acid-hydrolyzed fractions was, however, greatly in-

* Supported in part by a grant from the National Research Council of Canada.

creased above that observed in the non-pregnant female. In some of the urines tested, β-glucuronidase released only small additional amounts of aldosterone, whereas a subsequent acid hydrolysis at room temperature showed that relatively large quantities of this hormone were still present in the urine. The addition of desoxyribonucleic acid to pregnancy urine

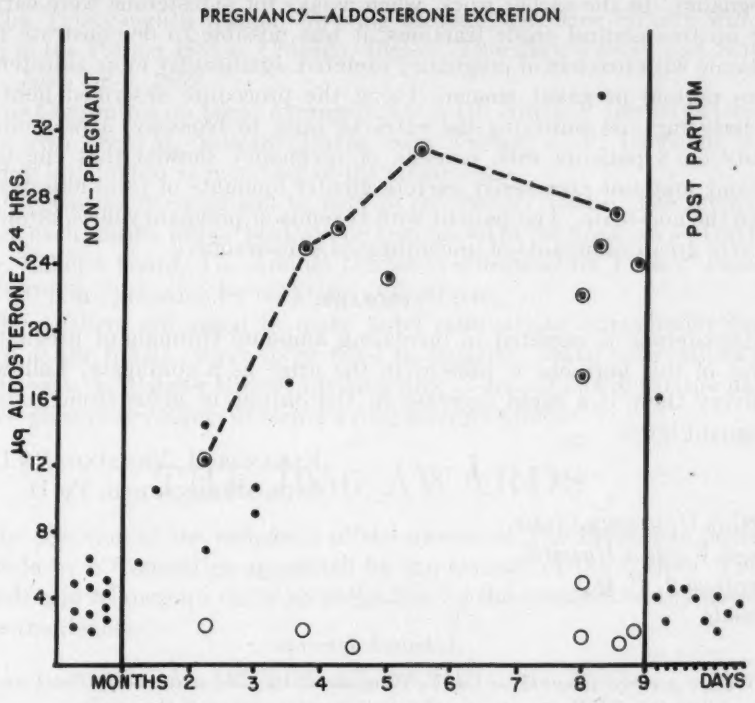

FIG. 1. Aldosterone excretion in pregnancy. ○ Free fraction only. • Free and acid-hydrolyzed fractions. ⊙ Free, enzyme, and acid-hydrolyzed.

prior to incubation with β-glucuronidase in some instances caused greater amounts of the aldosterone conjugate to be hydrolyzed.

Urines obtained from 7 pregnant women at various times in pregnancy were analyzed for the total content of aldosterone, using β-glucuronidase and acid hydrolysis. The values are charted in Figure 1 and are compared to those obtained in non-pregnant women and in women in the post-partum period.

After the third month there was a significant rise in the excretion of aldosterone, which remained at a high level up to term. The average output in the last month of pregnancy was 25 μg. per twenty-four hours. The curve of excretion of this hormone was followed in 1 case from the third month

until the second week before term. The output of aldosterone ranged from 12 μg. to 30 μg. per day (Fig. 1). Following delivery there was a rapid decrease in the excretion of aldosterone to non-pregnant levels.

The significance of these findings must be reconsidered in relation to the increased excretion of aldosterone observed in patients with toxemia of pregnancy. In the earlier work, when assays for aldosterone were carried out on free neutral crude fractions, it was possible to demonstrate that patients with toxemia of pregnancy excreted significantly more aldosterone than normal pregnant women. Using the procedure described here for hydrolyzing and purifying the extracts prior to bioassay, a preliminary study on 3 patients with toxemia of pregnancy showed that the toxic patient may not necessarily excrete greater amounts of total aldosterone than the non-toxic. The patient with toxemia of pregnancy does, however, excrete greater amounts of unconjugated aldosterone.

SUMMARY

Aldosterone is excreted in increasing amounts throughout pregnancy. Most of this hormone is present in the urine as a conjugate. Following delivery there is a rapid decrease in the output of aldosterone to non-pregnant levels.

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Acknowledgments

We are greatly indebted to Dr. T. Primrose of the Department of Obstetrics and Gynecology, McGill University, for the clinical material used in this study.

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The Endocrine Society

THE 1956 ANNUAL MEETING

The Thirty-eighth Annual Meeting of The Endocrine Society will be held in the Palmer House, Chicago, Illinois, Thursday, Friday and Saturday, *June 7-8-9, 1956.*

The Committee on Local Arrangements is Dr. Norris J. Heckel as Chairman with Drs. M. Edward Davis, Sam Taylor, J. P. Greenhill, and Harley E. Cluxton as members of the Committee.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Sey-

mour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

THE EIGHTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY; THE UNIVERSITY OF TEXAS POSTGRADUATE SCHOOL OF MEDICINE; AND THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, HOUSTON, TEXAS

October 22-27, 1956

The faculty will consist of twenty-two eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialities who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program and registration, write to *Dr. J. B. Trunnell, M. D. Anderson Hospital, 6723 Bertner Avenue, Houston 25, Texas.* Registration is limited to 100; tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Shamrock Hilton Hotel which is adjacent to the Texas Medical Center.

The American Goiter Association

THE 1956 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the Drake Hotel, Chicago, Illinois, May 3, 4 and 5, 1956.

1956 LAURENTIAN HORMONE CONFERENCE

The 1956 Laurentian Hormone Conference of the AAAS will be held at Mont Tremblant Lodge, Mont Tremblant, Quebec, during the period *September 2–7*, and investigators interested in attending this conference should make application to the Committee on Arrangements of The Laurentian Hormone Conference, 222 Maple Avenue, Shrewsbury, Massachusetts, at an early date and in any event no later than *May 7, 1956*. A conference rate of $12.00 per person per day is extended to all invited participants. Attendance is limited to invitations issued by the Committee on Arrangements. (The program will appear in a subsequent issue of this journal.)